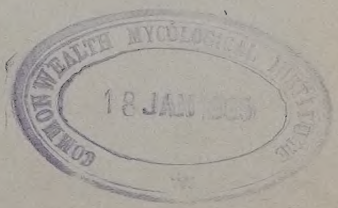
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Evolution and Variation in *Trillium*. IV.

Chromosomal Variation in Natural Populations of *Trillum kamtschaticum* Pall.*

By

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(Received April 21, 1956)

Introduction

The present investigation is an extension of the work carried out by Haga and Kurabayashi ('54), They found structural changes in chromosomes of *T. kamtschaticum* growing in Japan by means of differential reaction in chromosomes of this plant. Numerous different types were distinguished in each of the five chromosome pairs of this plant on the basis of the difference of the patterns in chromosome segments which revealed undercharging of nucleic acid in the differential reaction (Darlington & La Cour '38, '40, Haga & Kurabayashi '53, '54, Kurabayashi '52, etc.).

Analyses of the chromosome composition of natural populations of this plant were carried out by making distinctions of the chromosme types possessed by the individuals in each of the populations. The results obtained up to the year 1950 were presented in the previous paper (Haga & Kurabayashi '54). The principal features of evolutionary changes taking place among the natural populations of this plant were made clear by the analyses. Investigations on the same line were continued by the present writer with the cooperation of many researchers of our institute. The populations investigated up to date were chosen from so various localities as to cover nearly all the distribution areas of this plant in Japan (Fig. 1). In such circumstances, it seems necessary again to comment upon the evolutionary process of this plant in Japan.

Local distribution of various types of each chromosome arm

The numbers of types of different patterns found in each chromosome arm under the differential reaction were, up to the year 1953, 29, 11, 10, 7, 15, 16 and 4 as to both arms together of chromosome A, to the short arm of chromosomes B and E and to the long arm of chromosomes B, C, D and E respectively (Fig. 2). The short arms of chromosomes C and D were omitted from the analysis because of the absence of variation consistently detectable with ease and accuracy (cf. Haga & Kurabayashi '53, '54, Kurabayashi '52).

* Aided by Grants-in-Aid from the Ministry of Education in Japan.

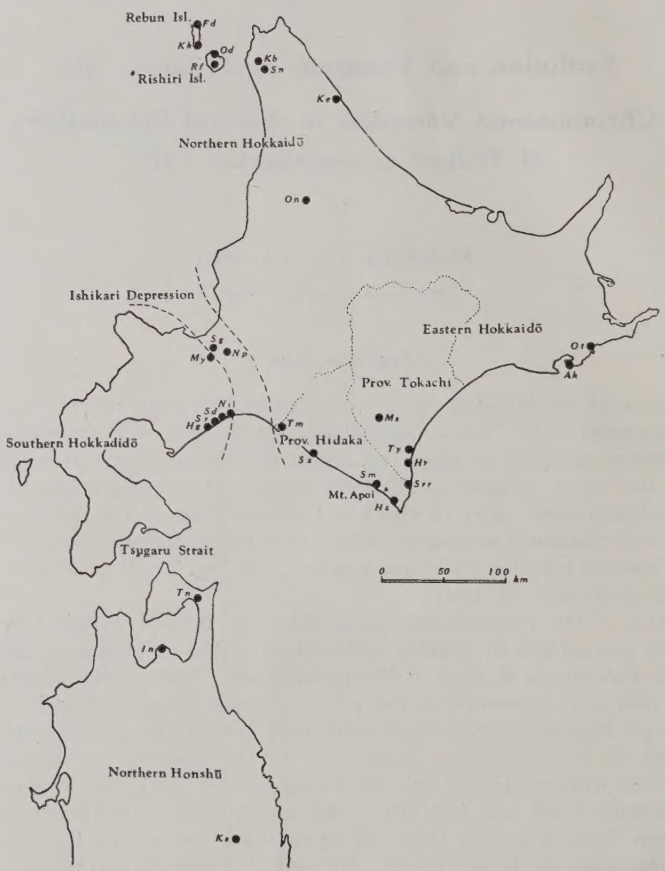

Fig. 1. A map of northern Japan showing the localities from which the natural populations of *T. kamtschaticum* investigated in the present paper were taken. The belt bordered by the broken lines is the Ishikari Depression. Provinces are shown enclosed by dotted lines. The name of each locality is shown abbreviated in the map. This abbreviated symbol is used in the text as the name of the population chosen for investigation in the corresponding locality (cf. Tables 1–5). The full name of each locality is shown in the Table of next page.

Two arms of chromosome A: This chromosome is iso-brachial in non-differentiated condition. In differentiated condition it reveals undercharging of nucleic acid at the proximal regions in the two arms. The two arms were sometimes distinguishable from each other based on the difference in the pattern of the proximal regions. Sometimes, however, they were indistinguish-

Abbrev. Symbol	Name of Locality	Abrrev. Symbol	Name of Locality
Ks	Kusakai	**Ms**	Minamisatsunai
In	Ino	**Ak**	Akkeshi
Tn	Tanabu	**Ot**	Ochiishi
Hg	Hagino	**Fd**	Funadomari
Sr	Shiraoi	**Kk**	Kafuka
Sd	Shadai	**Rf**	Rishirifuji
Ni	Nishikioka	**Od**	Oshidomari
Tm	Tomikawa	**Kb**	Kabutonuma
Sz	Shizunai	**Sn**	Shimonuma
Sm	Samani	**Ke**	Kitamiesashi
Hz	Horoizumi	**On**	Onnebetsu
Srr	Meguro	**Np**	Nopporo
Hr	Hiro	**Sg**	Sapporo-Genshirin
Ty	Toyoni	**My**	Maruyama

able, both having nearly identical differential patterns. Accordingly it was impossible to distinguish the right and left arm by simple comparisons of the patterns of the arms of an individual chromosome. An attempt was made at first to classify the arm patterns making no distinction as to the right and left arm, into four groups on the basis of the number of knobs and dots which locate intercalarily in the proximal differential segments and reveal no undercharging of nucleic acid in the differential reaction (Fig. 2, A). Based on this classification, the twenty-nine arm types of this chromosome were designated as follows: **101, 102, 103** and **104** as to the arms with one knob or dot; **201, 202,** .....and **213** as to those with two knobs or dots; **301, 302,**.....and **310** as to those with three; and **401** and **402** to those with four. The frequency and distribution of these types among the natural populations investigated are represented in Table 1 (cf. Appendix Table 1).

As indicated in this Table, the twenty-nine types did not distribute at random among the populations investigated. The maximum number of arm types found in one and the same population was twenty-two in **Ty** population while the minimum was two in **Ks, In, Hg, Sd, Ni, Tm, Fd, Kk, Rf, Ke** and **On** respectively (cf. Table. 1). Such difference in number of types included in each population roughly corresponded to the difference in population size, i.e., the larger the size of a population was, the larger, usually, was the number of the types included. The frequency of each arm type differed from population to population. It was noticed, however, that several types which were frequent in a given population were also found popular among the neighboring populations.* For example, there were thirteen types with frequency values of more than ten per cent in any one of **Sz, Sm, Srr(S), Srr(N), Ty, Ms,**

* The order of the populations written in Tables 1–5 follows the mutual relation in their geographical positions (cf. Fig. 1) except that between **Ot** and **Fd**, which are separated by double lines in the Tables.

Table 1. Type and frequency (%) of two arms of chromosomes A found in natural populations of *T. kamtschaticum* investigated.

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Srr (S)*	Srr (N)	Ty
Type Number													
101	—	100	38	—	—	—	100	—	—	—	1	1	—
102	—	—	1	—	—	—	—	—	—	—	1	1	2
103	—	—	—	—	—	—	—	100	4	1	—	—	1
104	—	—	—	—	—	—	—	—	—	—	11	13	5
201	—	—	—	—	—	—	100	100	71	53	44	45	37
202	—	—	—	—	—	—	—	—	14	2	1	—	3
203	—	100	47	—	—	—	—	—	1	3	27	24	23
204	100	—	52	(100)	100	(100)**	—	—	13	37	8	8	12
205	100	—	61	(100)	44	(100)	—	—	5	15	3	3	15
206	—	—	—	—	—	—	—	—	—	—	—	2	7
207	—	—	—	—	56	—	—	—	16	10	11	10	20
208	—	—	1	—	—	—	—	—	27	30	23	18	27
209	—	—	—	—	—	—	—	—	—	—	—	—	1
210	—	—	—	—	—	—	—	—	—	—	—	—	2
211	—	—	—	—	—	—	—	—	6	1	—	—	2
212	—	—	—	—	—	—	—	—	—	9	1	2	3
213	—	—	—	—	—	—	—	—	—	—	—	—	—
301	—	—	—	—	—	—	—	—	—	—	—	1	1
302	—	—	—	—	—	—	—	—	1	10	—	—	4
303	—	—	—	—	—	—	—	—	—	—	—	1	—
304	—	—	—	—	—	—	—	—	—	—	—	1	—
305	—	—	—	—	—	—	—	—	—	2	1	—	2
306	—	—	—	—	—	—	—	—	25	20	66	70	24
307	—	—	—	—	—	—	—	—	3	5	—	—	1
308	—	—	—	—	—	—	—	—	14	2	1	—	3
309	—	—	—	—	—	—	—	—	—	—	—	—	2
310	—	—	—	—	—	—	—	—	—	—	—	—	3
401	—	—	—	—	—	—	—	—	—	—	—	—	1
402	..... (Found once in a population at Horoizumi (**Hz**), Hidaka												
Total	200	200	200	(200)	200	(200)	200	200	200	200	199	200	201

* Two populations, **Srr** (**S**) and **Srr** (**N**), were chosen for investigation at Meguro, Hidaka province, Hokkaido (cf. Fig. 1). They were found respectively along the southern and northern side of the river Saruru.

** The frequency values put in parentheses were taken from the values obtained by the analysis of the natural populations of *T. Hagae* (3x) which grew mixed with *T. kamtschaticum* in the corresponding localities respectively (Haga '56).

Table 1. (continued to the right of the preceding page.)

Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
—	1	3	—	—	—	—	—	—	—	—	115	146	175
—	4	4	—	—	—	—	—	—	—	—	—	2	—
—	—	1	100	—	100	96	80	60	100	100	15	10	10
—	3	1	—	—	—	—	—	—	—	—	—	—	—
50	39	26	100	100	100	100	100	100	100	100	65	22	—
8	5	13	—	—	—	—	—	—	—	—	—	—	—
17	15	4	—	—	—	—	—	—	—	—	—	—	—
11	9	18	—	—	—	—	—	—	—	—	—	—	—
56	9	1	—	—	—	—	—	—	—	—	—	—	—
3	13	18	—	—	—	—	—	—	—	—	—	—	—
17	35	14	—	100	—	4	20	10	—	—	—	16	—
14	10	5	—	—	—	—	—	—	—	—	—	4	—
—	—	—	—	—	—	—	—	—	—	—	—	—	15
—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	3	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	30	—	—	5	—	—
—	13	46	—	—	—	—	—	—	—	—	—	—	—
3	5	1	—	—	—	—	—	—	—	—	—	—	—
—	1	2	—	—	—	—	—	—	—	—	—	—	—
—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	1	2	—	—	—	—	—	—	—	—	—	—	—
16	18	37	—	—	—	—	—	—	—	—	—	—	—
—	2	2	—	—	—	—	—	—	—	—	—	—	—
6	2	2	—	—	—	—	—	—	—	—	—	—	—
—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	9	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
Province, Hokkaido).....													
201	200	200	200	200	200	200	200	200	200	200	200	200	200

Ak and **Ot** population. Among the types, seven were found to be common in all, two in seven, one in six, two in five and one in three of the eight populations. The frequency of each of the thirteen types varied from population to population. As a rule, when the degree of variation was smaller, the average frequency of a common type in the populations was larger. That is, these neighboring populations, in spite of their wide range of variation in

Table 2. Type and frequency (%) of short (B–S) and long (B–L) arms of choromosome B.

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Srr (S)	Srr (N)	Ty
Type Number of B–S													
101	—	86	49	—	9	—	—	—	—	—	—	—	3
102	—	—	—	—	62	—	—	—	—	—	—	—	1
104	—	—	—	—	—	—	—	100	—	—	—	—	—
201	—	—	1	(100)	11	—	100	—	31	63	54	53	61
202	—	—	23	—	18	(100)	—	—	—	1	—	—	—
204	—	—	—	—	—	—	—	—	—	—	—	—	—
205	—	—	—	—	—	—	—	—	—	—	—	—	1
301	—	—	—	—	—	—	—	—	57	33	46	42	34
302	100	14	27	—	—	—	—	—	1	1	—	—	—
401	—	—	—	—	—		—	—	6	2	—	—	—
402	—	—	—	—	—	—	—	—	5	—	—	—	—
Total	100	100	100	(100)	100	(100)	100	100	100	100	100	100	100
Type Number of B–L													
50	100	100	99	(100)	18	(100)	100	—	100	100	100	100	96
151	—	—	—	—	61	—	—	—	—	—	—	—	1
152	—	—	—	—	9	—	—	—	—	—	—	—	—
153	—	—	—	—	—	—	—		—			—	—
251	—	—	—	—	8	—	—	—	—	—	—	—	—
252	—	—	1	—	4	—	—	—	—	—	—	—	3
253	—	—	—	—	—	—	—	100	—	—	—	—	
Total	100	100	100	(100)	100	(100)	100	100	100	100	100	100	100

chromosomal composition, had as many as eight predominant types in common.

Tn population included six arm types. Among them four were frequent and two were rare. Two of the frequent types were found in four of the neighboring populations, **Ks, Hg, Sr** and **Sd.** Another neighboring population, **In,** had the remaining two of the frequent types in fixed condition.

All the populations hitherto investigated in northern Hokkaido (**Fd, Kk, Rf, Od, Kb, Sn, Ke** and **On** in Table 1) had one common type in fixed condition. Another type was found fixed or in high frequency in all these population except one, **Kk.** Fixations of the arm types to a few common ones were also seen in middle Hokkaido (**Ni, Tm, Np, Sg** and **My**) where two of the three types (**101, 103** and **201**) were found fixed or in high frequencies in every populations. It was noticed, however, that one of the three types (**101**) was found in unexpectable frequencies, viz, more than 100 per cent, in **Np, Sg** and **My**

Table 2. (continued to the right of the preceding page.)

Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
—	6	6	—	—	100	—	9	—	7	—	52	34	100
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	32	96	—	—	—	—	—	—	—	—	—
64	29	35	68	4	—	19	26	80	79	45	45	64	—
—	—	1	—	—	—	—	—	—	—	—	—	—	—
—	3	2	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
36	40	27	—	—	—	81	65	20	14	55	3	2	—
—	22	29	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
100	100	100	100	100	100	100	100	100	100	100	100	100	100
100	100	100	68	4	100	100	100	100	93	100	40	4	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	3	2	10
—	—	—	—	—	—	—	—	—	7	—	50	32	90
—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	40	—	—	—	—	—	—	8	62	—
—	—	—	32	56	—	—	—	—	—	—	—	—	—
100	100	100	100	100	100	100	100	100	100	100	101	100	100

population. It must be remembered here that the two arms of chromosome A are being dealt with jointly, making no distinction between the right and left. Distinction is impossible, as desribed above, by simple comparison of the arm patterns. In the case of the middle Hokkaido populations, more than 50 per cent of chromosome A's were isomorphic in the differential patterns of the two arms (**101** type), thus naturally giving this type a frequency of more than 100 per cent. It is evident from this that some way must be found to distinguish the right and left arms of this chromosome before it will become possible to make comparisons of chromosomal compositions of natural populations of this plant. Such distinction was made fairly successfully by the analysis of the arm combinations (*vide infra*).

Short and long arms of chromosome B: The types hitherto found in these arms were designated as follows: **101, 102, 104, 201, 202, 204, 205, 301, 302, 401,**

402 (Fig. 2, B–S); **50, 151, 152, 153, 251, 252, 253** (Fig. 2, B–L). Here the type number of them is given following the same method as applied in chromosome A. To make the distinction between the short and the long arm, the type number for each of the latter was increased by fifty over that of the former. The type number **50** was given to the long arm type without any differential segment. The type and frequency of the two arms in each population are shown in Table 2 (cf. Appendix Table 2).

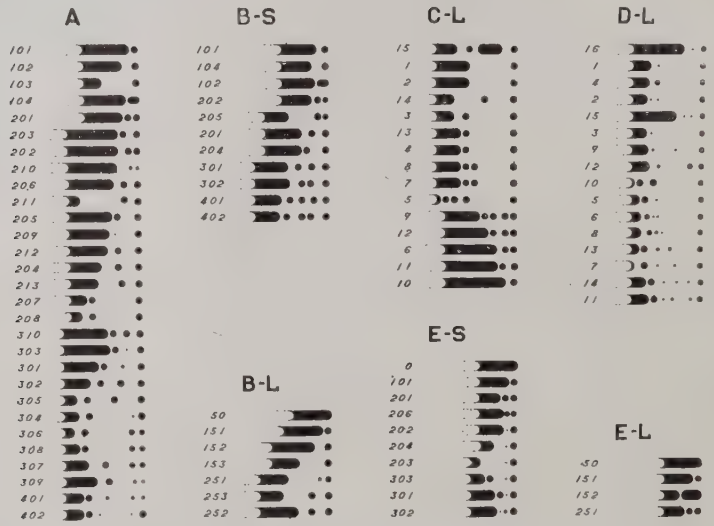

Fig. 2. Schematic representation of the types found in the two arms of chromosome A(A), in the short arms of chromosome B(B–S) and E(E–S), in the long arms of chromosome B(B–L), C(C–L), D(D–L) and E(E–L) respectively. They are arranged in the order of their resemblance in the differential patterns so far as the linear disposition allows.

A total of ten types of long arm were detected among **Sz, Sm,**.....and **Ot** population. Seven of these types were found only rarely in a few of these populations, two distributed in all the above mentioned populations with frequencies of more than 20 per cent, and the remaining one was detected in four of the populations with the frequency of 1, 1, 22 and 29 per cent respectively.

Three types of the long arm were found among **Rf, Od, Kb, Sn, Ke, On, Np, Sg** and **My** population. Fixation to one of the three types was attained in two of the nine populations (**Rf** and **My**); two of the three types co-existed with fairly high frequencies in three (**Od, Sn** and **On**); and in others, all of the three types were found.

There were four types of short arm in **Tn** population. Among them the next frequent was found fixed in **Ks** population and the most and next frequent ones co-existed in **In** population. Four short arm types were found in **Sr** popu-

lation. Three of them were identical with three of the four types found in **Tn,** while the remaining one was found in none of the neighboring populations. **Hg, Sd** and **Ni** populations respectively had one of the four types found in **Sr** in fixed condition. **Tm** population, which locates next to **Ni,** had one type which was identical with a type of short arm found only in **Fd** and **Kk** population in fixed condition.

One type of the long arm of chromosome B (type number **50**) had predominant frequencies in almost all the populations investigated. It was found fixed or nearly so except in seven populations: **Sr, Ty, Fd, Kk, Np, Sg** and **My**. **Np** and **Sg** respectively had four types in common. Among them two were found in **My** and three in **Sr.** In the latter, additional to the three, two types were found which were inherent to **Sr** population. **Kk** had three types, of which two were identical with two of the four found in **Np** and **Sg.** The remaining one was detected in **Fd** and predominant in **Tm.**

Long arm of chromosome C: Fifteen types were found among the populations investigated (Table 3, Fig. 2, C–L, and Appendix Table 3). Only one out of the ten types found among **Srr(S), Srr(N)**.....and **Ot** population predominated there over others. Two of the ten types had more or less higher frequencies in five, and the remaining seven were found only rarely in one to three of these populations.

Three types co-existed in **Tn** population. The one with the lowest frequency was found in none of the neighboring populations, the next occurred in the nearest, **In,** and the most frequent type was found fixed or nearly so in all the populations of northern Honshu, southern Hokkaido,* and also in **Ni** and **Tm.**

Nine types were found in common in **Sz** and **Sm.** The frequency of these types was not much different between the two populations. Five of the types had fairly high frequency and the remaining four were rather rare. Two of the frequent types were the identical ones which were common among the eastern Hokkaido populations, one was the same with that predominating among the northern Honshu and southern Hokkaido, and the remaining two were found though in low frequencies, in several of the eastern Hokkaido populations. Two of the rare types were found in some of the populations of one or both of the above mentioned districts, and the remaining two were unique ones never detected in other populations.

Kk population had four types. The one with the lowest frequency was never detected in other populations. One, two or all of the remaining three were found, sometimes fixed and sometimes co-existing among the populations

* For convenience' sake, the populations investigated were grouped, according to the name of the districts where they distribute, into five: 1) northern Honshu.....**Ks, In** and **Tn**; 2) southern Hokkaido.....**Hg, (Sr), Sd, (Ni)** and **(Tm)**; 3) eastern Hokkaido..... **(Sz), (Sm), Srr(S), Srr(N), Ty, Ms, Ak** and **Ot**; 4) northern Hokkaido.....**Fd, Kk, Rf, Od, Kb, Sn, Ke** and **On**; and 5) middle Hokkaido.....**Np, Sg** and **My**. The five populations included in parenthesis: **Sr, Ni, Tm, Sz** and **Sm** were sometimes treated separately or put into some of the other groups because of their peculiar chromosomal compositions (see the Text and Fig. 1).

Table 3. Type and frequency of long arms of chromosome C.

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Srr (S)	Srr (N)	Ty
Type Number													
1	—	—	—	—	—	—	—	—	16	7	6	32	5
2	—	—	—	—	—	—	—	—	12	8	—	—	1
3	—	—	1	—	—	—	—	—	38	31	53	51	90
4	—	—	—	—	—	—	—	—	2	6	—	—	—
5	—	—	—	—	—	—	—	—	10	22	—	—	1
6	100	95	73	(100)	100	(100)	100	100	16	16	—	—	—
7	—	—	—	—	—	—	—	—	4	2	—	—	—
8	—	—	—	—	—	—	—	—	1	7	—	—	—
9	—	5	27	—	—	—	—	—	1	1	—	—	2
10	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—	39	17	1
14	—	—	—	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—	2	—	1
Total	100	100	101	(100)	100	(100)	100	100	100	100	100	100	101

Table 4. Type and frequency of long arms of chromosome D.

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Srr (S)	Srr (N)	Ty
Type Number													
1	—	—	—	—	—	—	—	100	20	7	—	—	4
2	—	—	—	—	—	—	—	—	12	8	—	—	—
3	100	100	100	(100)	27	(100)	100	—	3	2	—	—	—
4	—	—	—	—	—	—	—	—	6	2	—	—	4
5	—	—	—	—	—	—	—	—	47	26	100	100	90
6	—	—	—	—	—	—	—	—	2	39	—	—	—
7	—	—	—	—	73	—	—	—	9	14	—	—	1
8	—	—	—	—	—	—	—	—	1	2	—	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—
12–14	..... (These three types were found in a population at												
15	—	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	100	100	100	(100)	100	(100)	100	100	100	100	100	100	99

Table 3. (continued to the right of the preceding page.)

Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
—	10	5	–	–	—	—		—	—	—	—	—	
—	3	—	—	—	—	—	—			–	—	–	—
92	62	75	—	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	–		–	–	—	
3	5	—	—	—	–		–	—	–	—	—	—	—
—	—	—	—	19	7	15	79	80	100	100	83	90	30
—	—	—	–		–	–	—	–		–	—	—	–
	—	–	–	—	–		–	—	—	—	—	–	
—	2			—		—	—	—		—	–	–	
—	—	—	100	48	93	85	21	20	—	—	3	—	—
—	1	—	—	31	—	—	—	—	—	—	15	10	70
	—	–	—	2	—	—	—	—	—	—	—	—	—
—	16	20	—	–	–	—	–	–	–		–	–	
—	1	—	—	—	–		–	—	–	—	—	—	–
—	—	—	—	—	—	—	—	—	—	—	—	—	—
101	100	100	100	100	100	100	100	100	100	100	101	100	100

Table 4. (continued to the right of the preceding page.)

Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
—	20	9	100	100	100	100	100	100	100	100	45	70	40
–	—	—	—	—	—	—	—	—		–	–	—	—
—	—	—	—	—	—		—	—	–	–	55	—	10
6	1	1	–	—	—	–	—	—	—		–	–	–
94	76	90	—	–	–	—	–	–	–		—	—	—
—	—	–	—	–	–			—	—		–	—	—
—	1	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	–	–	–	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	4	—
—	1	—	—	—	—	—	–	—	—	—	–	—	—
—	1	—	–		—	—	—	—	—	—	—	—	—
Horoizumi)													
–	—	—	—	—	—	—	—	—	—	—	—	22	45
—	—	—	—	—	—	—	—	—	—	—	—	4	5
100	100	100	100	100	100	100	100	100	100	100	100	100	100

Table 5. Type and frequency of short and long arms of chromosome E.

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Srr (S)	Srr (N)	Ty
Type Number of E-S													
0	—	—	—	—	—	—	—	—	—	—	—	—	4
101	—	—	52	—	—	—	—	—	4	9	16	11	49
201	—	—	9	(100)	100	(100)	—	—	9	6	—	—	—
202	—	100	25	—	—	—	100	—	65	67	39	41	5
203	—	—	1	—	—	—	—	—	22	18	16	30	3
204	—	—	—	—	—	—	—	63	—	—	29	19	39
206	—	—	—	—	—	—	—	—	—	—	—	—	—
301	100	—	13	—	—	—	—	—	—	—	—	—	—
302	—	—	—	—	—	—	—	38	—	—	1	—	—
303	—	—	—	—	—	—	—	—	—	—	—	—	1
Total	100	100	100	(100)	100	(100)	100	101	100	100	101	101	101
Type Number of E-L													
50	—	—	—	—	—	—	—	63	—	—	—	2	12
151	100	100	99	(100)	100	(100)	100	38	100	97	100	97	87
152	—	—	1	—	—	—	—	—	—	3	—	—	—
251	—	—	—	—	—	—	—	—	—	—	—	1	1
Total	100	100	100	(100)	100	(100)	100	101	100	100	100	100	100

in northern and middle Hokkaido (**Fd, Kk,**.....**Sg,** and **My**).

Long arm of chromosome D: There were sixteen types among the populations investigated (cf. Fig. 2, D–L, Table 4, and Appendix Table 4). The populations of northern Honshu and southern Hokkaido (**Ks, In, Tn, Hg, Sd** and **Ni**), eastern Hokkaido (**Srr(S), Srr(N), Ty, Ms, Ak** and **Ot**), and northern Hokkaido (**Fd, Kk, Rf, Od, Kb, Sn, Ke** and **On**) had respectively one predominant type which was common in each and different from the ones found in the other population groups just noted. A total of five types were found in **Np, Sg,** and **My.** Among them, one was identical with the type found predominantly in northern Honshu and southern Hokkaido and another was with that included in northern Hokkaido. The remaining three were unique types included only in **Sg** and **My** or in one of the two.

Two types were included in **Sr** population. One of them was identical with one found in northern Honshu and southern Hokkaido. The other type was detected in **Sz** and **Sm,** in which all the predominant types found in the above mentioned three population groups co-existed in fairly high frequencies. In addition to these another four types were included in **Sz** and **Sm**. One of the

Table 5. (continued to the right of the preceding page.)

Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
—	—	—	—	—	—	—	—	—	—	—	—	—	
34	16	30	—	—	—	—	—	—	—	—	35	14	70
—	5	—	—	—	—	—	—	—	—	—	—	—	—
56	41	36	—	—	—	—	—	—	—	—	28	6	—
8	25	—	—	—	—	—	—	—	—	—	—	—	—
3	5	34	—	—	—	8	41	100	100	100	38	80	30
—	6	—	—	—	—	—	—	—	—	—	—	—	
—	1	—	—	—	—	—	—	—	—	—	—	—	–
—	—	—	100	100	100	92	59	—	—	—		—	
—	1	—	—	—	—	—	—	—	—	—	—	—	—
101	100	100	100	100	100	100	100	100	100	100	101	100	100
—	10	—	—	—	—	8	17	10	—	—	—	—	—
100	88	100	100	100	100	92	83	90	100	100	100	100	100
—	—	—	—	—	—	—		—	—	—	—	—	—
—	2						—	—	—	—		—	—
100	100	100	100	100	100	100	100	100	100	100	100	100	100

four was found, though in low frequencies, in four of the eastern Hokkaido populations, while the remaining three were inherent to **Sz** and **Sm.**

Short and long arm of chromosome E: Total ten and four types found respectively in the short and the long arm of chromosome E distributed as indicated in Table 5 (cf. Fig. 2, E–S and E–L, and Appendix Table 5). Populations of **Srr(S)**, **Srr(N)**, **Ty, Ms, Ak** and **Ot** had three types of short arm in common. Another one type was found in fairly high frequencies in five of the six populations. The remaining six types found among these populations were very low in frequency and included only in a few of them.

Kb population had two types of the short arm in approximately equal frequencies. One of them was found fixed or nearly so in **Fd, Kk, Rf** and **Od,** and the other was in complete fixation in **Sn, Ke,** and **On.** These two types coexisted also in **Tm.**

Np and **Sg** population commonly had three types of the short arm. Two of the three with higher frequency were also found in **My.**

Five types were found in **Tn.** The neighboring populations (**Ks, In, Hg, Sr, Sd** and **Ni**) had respectively one of the three among these five types in fixed

condition. **Sz** and **Sm** had four types in common. All these types were popular ones in some of the neighboring populations.

Twenty-six out of the twenty-seven populations investigated had one and the same type, **151,** of the long arm in fixed or nearly fixed condition. The exceptional one, **Tm** population, included two types, **50** and **151,** with frequencies of 63 and 38 per cent respectively. The former type co-existed with the latter in **Srr(N), Od, Kb** and **Sn** with frequencies of 2, 8, 17 and 10 per cent respectively. In **Ty** and **Ak** type **50** was found together with two other types, **151** and **251.**

Geographically, **Tm** is located nearer to the populations of southern and eastern Hokkaido than to the northern (cf. Fig. 1). This population, however, had, as described above (cf. Tables 1–5), abundance of northern elements. Further, the type of the short arm combining with the **50** type of long arm found in **Tm** was the identical one found in northern Hokkaido populations and different from those found in eastern Hokkaido (cf. Appendix Table 5).* These facts suggest strongly that type **50** found in **Tm** belongs also to the northern element.

Combination of arms in chromosomes A, B and E

Chromosomes A, B and E respectively have two arms, right and left or short and long, which respectively reveal, in the majority of cases, differential reactions in the chilled condition. As the result of their combinations, various types of chromosomes were found. The number of types hitherto found was fifty-five, eighteen and seventeen as to chromosomes A, B and E respectively. The mode of the combination must be analysed in detail because it contributes to or magnifies the intra- and inter-populational chromosomal variation just described with respect to the arm types.

Combination between the short and the long arms of chromosome B: The number of combinations between the different types of short and long arms of chromosome B found in natural populations investigated was much smaller than that expected from the random occurrence of the combionation (Table 6). This is one thing which may be caused due to the predominance of one type of the short arm (type number **50**) in almost all the populations investigated. Another cause may be the extinction of chromosome types with various combinations from populations due to the effect of natural selection and random genetic drift. Both of these factors may in turn have brought about the predominance of **50** types.

Three types of long and five types of short arms were found in **Ty.** Only six out of the fifteen possible combinations were detected among one hundred and seventy-two chromosomes analysed (Table 7). This population was the largest one ever examined by the present writer. Even in such a population

* The combination identical with that found in **Tm** was met only once in **Srr(N)**. That may, however, be an accidental coincidence.

Table 6. Combinations between different types of short and long arms of chromosome B detected in natural populations investigated.

	Short Arm											
Arm Type	**101**	**102**	**104**	**201**	**202**	**204**	**205**	**301**	**302**	**401**	**402**	Total
Long Arm												
50	250	—	—	517	40	5	—	410	191	8	5	1426
151	—	62	—	—	—	—	1	—	—	—	—	63
152	14	—	—	—	—	—	—	2	—	—	—	16
153	55	—	—	—	—	—	—	—	—	—	—	55
251	—	1	—	7	—	—	—	—	—	—	—	8
252	—	—	26	55	—	—	—	—	—	—	—	81
253	—	—	35	—	—	—	—	—	—	—	—	35
Total	319	63	61	579	40	5	1	412	191	8	5	1684

Table 7. Combinations between short and long arms of chromosome B in **Ty** population.

	Short Arm					
Long Arm	**101**	**102**	**201**	**205**	**301**	Total
50	6	—	100	—	59	165
151	—	1	—	1	—	2
252	—	—	5	—	—	5
Total	6	1	105	1	59	172

the predominance of **50** type was so conspicuous that there remained few traces of recombination of arms to result in the formation of new type chromosomes.

There were four and five types of short and long arm in **Sr.** Predominance of any one of these types was not very conspicuous as compared with **Ty.** However, the number of arm combinations was again far smaller than that of the possible number (Table 8). There were three types of chromosomes with the arm combinations of **102–151, 201–251** and **102–251** with the frequency of sixty-one, seven and one respectively out of one hundred chromosomes analysed (Table 8). None of these arm types had ever previously been found in the neighboring populations. Accordingly, the previous assumption (Haga & Kurabayashi '54) becomes plausible that the type **102–251,** which was found only once in **Sr** population, had arisen in that population due to the recombination between the short and long arm of the two chromosomes with the arm types of **102–151** and **201–251** respectively.

The short arm **104** combined with **252** in **Tm,** while the former combined with **252** in **Fd.** Both the arm combinations co-existed in **Kk.** Three and four types of short arms were found in **Np, Sg** and **My.** There were, however, only five (**101–152, 101–153, 201–50, 201–252** and **301–50**) out of twelve possible com-

Table 8. Combination between the short and long arms of chromosome B detected in **Sr** population.

	Short Arm				
Long Arm	**101**	**102**	**201**	**202**	Total
50	—	—	—	18	18
151	—	61	—	—	61
152	9	—	—	—	9
251	—	1	7	—	8
252	—	—	4	—	4
Total	9	62	11	18	100

binations (Table 9). The combinations found in **Np** and **Sg** completely coincided with each other. Two of them were retained in **My.** One of the five arm combinations, viz., **101–153,** which was popular among **Np, Sg** and **My** was found once in **Ke.**

Combination between the short and long arm of chromosome E: Seventeen out of forty possible combination have been detected up to date (Table 10). One type of long arm, **151,** was fixed in eighteen out of twenty-seven populations investigated (Table 5). This type attained from 83 to 99 per cent in eight of the remaining populations. Only in one population **Tm,** did it co-exist with another type, **50,** which attained the frequency of 63 per cent.

Table 9. Distribution of the detected combination in **Np**, **Sg** and **My**.

	Population		
Detected Combination	**Np**	**Sg**	**My**
101—152	+	+	+
101—153	+	+	+
201— 50	+	+	−
201—252	+	+	−
301— 50	+	+	−

The four populations, **Srr(N), Hr** (Appendix Tables 1–5 and Fig. 1) **Ty** and **Ak** included type **50** in the following combinations: **101–50** and **203–50** in **Srr (N); 206–50** in **Hr; 0–50, 101–50, 203–50** and **303–50** in **Ty**; and **101–50, 203–50** and **303–50** in **Ak.** It is worthy of note that type **50** combined in these populations, which were large in size, with the three types of short arm, **0, 206** and **303,** which were all rare types found restrictedly in one or two of the four populations, and that they never meet with any other short arm than **50** (cf. Table 5 and Appendix Table 5). This fact decreases the possibility that the just mentioned three types of chromosomes **0–50, 206–50** and **303–50** were derived from recombinations of right and left arms between certain pairs of chromosomes such as **x–50** and **O–y, x′–50** and **206–y′** or **x″–50** and **303–y″** (x, x', x'', y, y' and y'' respectively represents one of the popular types of short (x, x' and x'') long (y, y' and y'') arm of chromosome E found among eastern Hokkaido populations).

Table 10. Combinations between different types of short and long arms of chromosome E detected in natural populations investigated.

	Short Arm										
Long Arm	**0**	**101**	**201**	**202**	**203**	**204**	**206**	**301**	**302**	**303**	Total
50	6	10	—	—	8	14	7	—	—	3	48
151	—	258	126	525	117	271	—	112	218	—	1627
152	—	—	—	2	2	—	—	—	—	—	4
251	—	—	—	4	1	—	—	—	—	—	5
Total	6	268	126	531	128	285	7	112	218	3	1684

Two types of short and long arms co-existed in **Tm, Od** and **Kb.** The two short arm types were found, in **Kb,** in combination with three types of long arm, viz., **204–50, 204–151** and **302–151.** The latter two combinations were detected in **Tm** and **Od** also. Another possible combination, **302–50,** has not yet been found in any of the populations investigated (cf. Appendix Table 5).

The examinations of arm combinations described above yielded few direct proofs for or against the occurrecne of recombinations among the short and long arms of chromosomes B and E to result in the formation of new chromosome types. It is certain, however, that the recombinations, if possible, occur so rarely and so restrictedly in these chromosomes that only fractions of possible combinations are kept alive even in large populations investigated (cf. Tables 6 and 10 and Appendix Tables 2, 5).

Combination between the right and left arms of chromosome A: This chromosome, as described before, has two morphologically indistinguishable arms. This situation is quite unfavorable for the analyses of chromosomal composition of natural populations because it is impossible to distinguish a pair of chromosome A's with an identical pair of arm types reversely in right and left, respectively. An attempt is made in the following to point out how a distinction may be made by way of comparative examinations of combinations of arm types detected up to the present.

At first, the frequency of combinations of arm types of this chromosome detected in Hidaka and eastern Hokkaido populations (**Sz, Sm, Hz, Srr(S), Srr (N), Hr, Ty, Ms, Ak** and **Ot**) is shown in Table 11 making no distinctions as to the right and left arms. These populations were large and nearly continuous as indicated by the types of chromosomes B, C, D and E included among them (cf. Tables 2–5 and Appendix Tables 2–5). Forty-nine out of fifty-five detected types of chromosome A were included in these ten populations. These types had twenty-eight out of twenty-nine detected arm types in various combinations. The frequency of each arm type and each combination varied conspicuously as shown in this Table. Some of the arm combinations with larger possibilities of existence expressed by the product of the frequency of a given pair of arms taking part in a given combination (cf. the foot note to Table 11) were found much more frequently than those with smaller possibilities. At the same time,

Table 11. Combinations of arms of chromosome A in

Type Number	101	102	103	104	201	202	203	204	205	206	207	208	209	210
101	.*	.	.	.	.:	.	:	:	:	.	:	:	.	.
	—	—	—	—	2	—	—	—	—	—	—	4	—	—
102	.	.	.	:	::	:	.:	.:	.:	:	.:	::	.	.
	—	—	—	—	—	—	—	—	—	—	—	11	—	—
103	.	.	.	.	::	.	:	:	:	:	.:	::	.	.
	—	—	—	—	8	—	—	—	2	—	—	—	—	—
104	.	:	.	:	.::	.:	::	::	::	.:	::	::	.	.
	—	—	—	—	—	—	—	—	—	10	2	8	—	—
201	.:	::	::	...	:::	...	:::	:::	:::	...	:::	:::	:	.:
	2	—	8	—	—	—	—	—	61	30	46	62	—	—
202	.	:	.	.:	.::	.:	::	::	::	.:	::	::	.	.
	—	—	—	—	—	—	3	—	—	14	—	—	—	—
203	:	.:	:	::	:::	::	.::	.::	.::	::	.::	.::	.	:
	—	—	—	—	—	3	—	—	15	—	20	7	—	—
204	:	.:	:	::	:::	::	.::	.::	.::	::	.::	.::	.	.
	—	—	—	—	—	—	—	—	9	—	14	25	—	—
205	:	.:	:	::	:::	::	.::	.::	::	::	.::	.::	.	.
	—	—	2	—	61	—	15	9	—	—	—	—	—	—
206	.	:	:	.:	.::	.:	::	::	::	.:	::	.::	.	.
	—	—	—	10	30	14	—	—	—	—	—	—	—	—
207	:	.:	.:	::	:::	::	.::	.::	.::	::	.::	:::	.	:
	—	—	—	2	46	—	20	14	—	—	26	21	—	—
208	:	::	.:	::	:::	::	.::	.::	.::	.::	:::	:::	:	:
	4	11	—	8	62	—	7	25	—	—	21	24	1	—
209	.	.	.	.	:	.	.	.	.	.	.	:	.	.
	—	—	—	—	—	—	—	—	—	—	—	1	—	—
210	.	.	.	.	.:	.	:	.	.	.	:	:	.	.
	—	—	—	—	—	—	—	—	—	—	—	—	—	—
211	.	.	.	.	::	.	:	:	:	:	.:	.:	.	.
	—	—	—	—	8	—	—	3	—	—	—	—	—	—
212	.	.	.	:	::	:	.:	.:	.:	:	::	::	.	.
	—	—	—	—	—	—	—	21	—	—	—	—	—	—
301	.	:	:	.:	.::	.:	::	::	::	::	.::	.::	.	.
	—	—	—	—	—	—	—	—	—	—	—	—	—	—
302	.	.	.	:	.::	:	::	.:	.:	:	::	::	.	.
	—	—	—	—	13	—	12	—	—	—	—	—	—	—
303	.	.	.	.	::	.	:	:	:	.	:	:	.	.
	—	—	—	—	4	—	—	—	—	—	—	—	—	—
304	.	.	.	.	:	.	.	.	.	.	.	:	.	.
	—	—	—	—	—	—	—	—	—	—	—	—	—	—
305	.	.	.	.	::	.	:	:	:	:	.:	::	.	.
	—	—	—	—	—	—	—	—	—	—	—	10	—	—
306	.:	::	::	...	:::	...	:::	:::	:::	.::	:::	:::	:	:
	—	7	—	15	140	—	73	32	—	—	34	—	—	—
307	.	.	.	.	::	:	.:	.:	:	:	.:	.:	.	.
	—	—	—	—	—	—	—	13	—	—	—	—	—	—
308	.	:	.	:	.::	.	::	::	.:	.:	::	::	.	.
	—	—	—	—	—	28	—	—	—	—	—	2	—	—
309	.	.	.	.	.:	.	:	.	.	.	:	:	.	.
	—	—	—	—	—	—	—	—	—	—	—	—	—	4
310	.	.	.	:	::	:	.:	.:	:	:	.:	.:	.	.
	—	—	—	—	15	—	—	—	—	—	1	—	—	—
401	.	.	.	.	.	.	.	:	.	.	.	.	.	.
	—	—	—	—	—	—	—	1	—	—	—	—	—	—
402	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	—	—	—	—	—	—	—	—	—	—	—	—	1	—
Total	6	18	10	35	386	45	130	118	87	54	164	175	2	4

* The number of dots put above the frequency value of each combination represents the total frequency values of the two arms which relate with the combination. One, $1500<P<3000$, $3000<P<9000$, $9000<P<27000$ and $27000<P$, respectively.

the populations of Hidaka and eastern Hokkaido.

211	212	301	302	303	304	305	306	307	308	309	310	401	402	Total
.	.	.	.	.	.	.	.:	.	.	.	.	:.	.	6
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
.	.	:	.	.	.	.	::	.	:	.	.	.	.	18
—	—	—	—	—	—	—	7	—	—	—	—	—	—	
.	.	:	.	.	.	.	::	.	.	.	.	.	.	10
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
.	:	.:	:	.	.	.	.::	.	:	.	:	.	.	35
—	—	—	—	—	—	—	15	—	—	—	—		—	
::	::	.::	.::	::	:	::	:::	::	.::	.:	::	.	.	389
8	—	—	13	4	—	—	140	—	—	—	15	—	—	
.	:	.:	:	.	.	.	.::	:	:	.	:	.	.	45
-	—	—	—	—	—	—	—	—	28	—	—	—	—	
:	.:	::	::	:	.	:	:::	.:	::	:	.:	.	.	130
—	—	—	12	—	—	—	73	—		—	—	—	- -	
:	.:	::	.:	:	.	:	:::	.:	::	.	.:	.	.	118
3	21	—	—	—	—	—	32	13	—	—	—	1	—	
:	.:	::	.:	:	.	:	.::	:	.:	.	:	.	.	87
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
:	:	::	:	.	.	:	.::	:	.:	.	:	.	.	54
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
.:	::	.::	::	:	.	.:	:::	.:	::	:	.:	.	.	164
—	—	—	—		—		34	—	—	—	1	—	—	
.:	::	.::	::	:	:	.:	:::	.:	::	:	.:	.	.	175
—	—	—	—	—	—	10	—	—	2	—			—	
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—	—	58	—	4	—	—	1	—	—	—	—	-		
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.	—	4	—	—	—	—	—	—	—	—	.	—	—	
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.	.	:	.	.	.	.	::	.	.	.	.	.	.	10
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
::	::	.::	::	.:	:	::	:::	::	.::	:	::	.	.	305
—	—	1	—	—	3	—	—	—	—	—	—	—	—	
.	.	:	.	.	.	.	::	.	.	.	.	.	.	13
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—	—	—	—	—	—	—	—	—	—	—	—		—	
.	.	.	.	.	.	.	:	.	.	.	.	.	.	4
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—	—	—	—	—	—	—	—	—	—	—	—	—	—	
.	.	.	.	.	.	.	.	.	.	.	.	.	.	1
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
11	21	63	25	8	3	10	305	13	30	4	16	1	1	1750

the possibility of existence of the corresponding combination as measured by the product two, three, four, five and six dots are put for the product, P: $P<500$, $500<P<1500$.

there remained numerous arm combinations with large possibilities of existence undetected. Two types of arms, **201** and **306**, with the largest and the next to the largest frequencies respectively, were chosen for an examination of the mode of combination between them and also among the others with which both or one of the two entered into combination (Tables 12–14).

The most frequent type, **201**, combined with eleven other different types. About a dozen of arm combinations with large possibilities of existence remained undetected, however, by the present examination. Such was also the case of next frequent type, **306**, which united with eight other different arm types, but there remained undetected more than ten highly possible combinations (Table 11).

Combinations between the two arm type groups, each of which combined with **201** and **306** respectively, were scarcely met with except for the combinations to which the types **207, 208** and **301** related (cf. Tables 12, 13 and the following descriptions). If the possibility of existence of each combination is expressed by the number of dots as defined in the foot note of Table 11, only four out of one hundred and sixty-eight dots (2%) would be found to exist in the combinations among the types which met with **201** (excluding **207** and **208**), while forty-five out of one hundred and fifty-five (29%) could be realized in the combinations between the two, **207** and **208**, and the former (compare the inside with the outside of the crisscross in Table 12). No actual example of combination was found out of one hundred and thirty-eight possible dots relating to the combinations among the arm types which combined with **306** (excluding **207** and **301**). While forty-nine out of one hundred and seventeen (42%) were discovered in the combinations between the two (**207** and **301**) and the former (compare the inside with the outside of the crisscross in Table 13).

The combinations between the two groups of types, the one combining with **201** and the other with **306** (excluding **201, 207, 208, 301** and **306**), were found more often than those between the two groups of types (Table 14). The percentage of actual existence in the combinations between the two arm groups expressed by the number of dots was twenty-four.

Based on these findings, it may tentatively be assumed that there are three groups of arm types distinguishable from each other with respect to their mode of combination. The first group includes **101, 205, 206, 211, 302, 303, 306** and **310**. They combine with **201** but do not with **306** and with each other, while they meet frequently with the second group. The latter includes **102, 104, 201, 203, 204** and **304**. They unite with **306** but neither with **201** nor with each other. The third group includes **103,* 207, 208** and **301**. They combine not only with some of the members of both of the first and the second group, but also some times with themselves.

* The combination, **103–205**, was the only one in which **103** was found to meet with a type belonging to the first group (Table 12). This indicates that either one or both of these types may be taken both by the right and left arms of chromosome A. It was decided, however, that, with reference to other populations (cf. Tables 15, 16), **103** was taken by the both arms and **205** was not.

Now, let the first group be assumed as left arm (L), and the second be the right (R). The third, then, may be regarded as the group including types which are accepted in combination both by right and left arm (RL). The re-

Table 12. Combinations detected in the arms which unite with 201 in Hidaka and eastern Hokkaido populations.

Type Number	101	103	205	206	207	208	211	302	303	306	310
101	·	·	:	·	:	:	·	·	·	··	·
103	·	·	:	:	.:	.:	·	·	·	··	·
205	:	:	::	::	.::	.::	:	.:	:	.::	:
206	·	:	::	.:	:.	.::	:	:	·	.::	:
207	:	.:	.::	::	.::	.::	.:	::	:	:::	.:
208	:	.:	.::	.::	:::	:::	.:	::	:	:::	.:
211	·	·	:	:	.:	.:	·	·	·	··	·
302	·	·	.:	:	::	::	·	:	·	··	·
303	·	·	:	·	:	:	·	·	·	.:	·
306	.:	::	.::	.::	:::	:::	::	:.	.:	:::	::
310	·	·	:	:	.:	.:	·	·	·	··	·

Table 13. Combinations among the arms which unite with 306.

Type Number	102	104	201	203	204	207	301	304
102	·	·	::	.:	.:	.:	:	·
104	:	:	.::	::	::	::	.:	·
201	::	.::	:::	:::	:::	:::	.::	:
203	.:	::	:::	.::	.::	.::	::	·
204	.:	::	:::	.::	.::	.::	::	·
207	.:	::	:::	.::	.::	.::	.::	·
301	:	.:	.::	::	::	.::	::	·
304	·	·		·	·	·	·	·

maining arm types found in Hidaka and eastern Hokkaido populations were those which met neither with **201** nor **306.** Among them, **202** combined with types belonging both to R and L and so may be RL. Types **212, 307** and **401** were found to combine with R, and may accordingly be L.

Table 14. Combinations between the two groups of arms, the one combining with **201** and the other with **306** (excluding **201, 207, 208, 301** and **306**).

Type Number	101	103	205	206	211	302	303	310
102	· / —	· / —	.: / —	: / —	· / —	· / —	· / —	· / —
104	· / —	· / —	:: / —	.: / +	· / —	: / —	· / —	: / —
203	: / —	: / —	.:: / ⊦	:: / —	: / —	:: / +	: / —	.: / —
204	: / —	: / —	.:: / ⊦	:: / —	: / ⊦	.: / —	: / —	.: / —
304	· / —	· / —	· / —	· / —	· / —	· / —	· / —	· / —

Table 15. Arm combinations found among the populations in northern Honshu and southern Hokkaido.

Population	Ks	In	Tn	Hg	Sr	Sd
R – L						
102–208*	—	—	+	—	—	—
203–101	—	+	+	—	—	—
203–205	—	—	+	—	—	—
204–205	+	—	+	+	+	+
204–207*	—	—	—	—	+	—

* Arm types which are grouped into RL in Hidaka and eastern Hokkaido populations.

Regarding others: **209, 210, 305, 308, 309** and **402**; it was impossible to determine to which arm they belong, because they met neither with R nor with L, but either with RL or with some one of the just described six types (cf. the seven in "?–RL", and eight in "?–?", columns of Table 17).

There were seven arm types of chromosome A in northern Honshu and southern Hokkaido populations (**Ks, In, Tn, Hg, Sr** and **Sd**). The arm combinations detected there were represented in Table 15. These arm types were grouped into R and L according to the same criteria as they were in Hidaka and eastern Hokkaido populations. The distinction between R and L was then completely possible in respect to the chromosomes found in the populations listed in Table 15.

Eight types were found in the middle and northern Hokkaido populations. There were two predominant types, **101** and **201.** The former combined with itself (**101–101**), and so must be RL. It also combined with five of the remain-

Table 16. Arm combinations found among the populations in middle and northern Hokkaido.

Population	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My	Ni	Tm
R-L													
101-101	—	—	—	—	—	—	—	—	+	+	+	—	—
**101-103*	—	—	—	—	—	—	—	—	—	—	+	—	—
**101-207*	—	—	—	—	—	—	—	—	—	+	—	—	—
**101-208*	—	—	—	—	—	—	—	—	—	+	—	—	
101-209*	—	—	—	—	—	—	—	—	—	—	+	—	—
102-208*	—	—	—	—	—	—	—	—	—	+	—	—	—
201-101**	—	—	—	—	—	—	—	—	+	+	—	+	—
201-103*	+	—	+	+	+	+	+	+	+	+	—	—	+
201-207*	—	+	—	+	+	+	—	—	—	—	—	—	—
201-213****	—	—	—	—	—	+	—	—	+	—	—	—	—

* Arm types groupd into RL in the above mentioned two population groups.

** Type **101**, which is grouped into L in the above population groups, is revised here to RL.

*** It cannot be decided to which arm groups type **209** belongs in the above population groups; it is regarded here to belong to L.

**** Type 213 is inherent to the populations listed in this Table.

Table 17. Arm Combinations in chromosome A represented making distinctions of the arm groups, R, L and RL respectively.

Arm Group	R - L	R - L	R - RL	RL - L	RL-RL	? - RL	? - ?
Detected Arm Combinations	102-306	203-205	102-208	103-205	101-101	209-101*	209-402*
	104-206	203-302	104-207	202-206	101-103*	209-208*	210-309*
	104-306	203-306	104-208	207-306	101-207*	305-208*	
	201-205	204-205	201-101	207-310	101-208*	308-202*	
	201-206	204-211	201-103	301-303	207-207	308-208*	
	201-211	204-212	201-207	301-306	207-208*		
	201-213	204-306	201-208		208-208		
	201-302	204-307	203-101		301-301		
	201-303	204-401	203-202				
	201-306	304-306	203-207				
	201-310		203-208				
			204-207				
			204-208				

* The eleven combinations marked with asterisks are those in which it is impossible to distinguish their arms are right or left, so that they may include two different types which respectively have the right and left arms in reverse combination from each other.

ing seven types including **201**. This makes it impossible to distinguish into R and L the arm types found in these populations. It was assumed for the time

being, that **201** is R, as it was so in the above described population groups (cf. Tables 12–15), and that the types which combine with it are L. By doing so the distinction of R and L could, for the nonce, be made (Table 16).

The above distinctions of right and left arms of chromosome A's were made rather *a priori*, or were made so as to divide the arms most conveniently and successfully into R and L. It followed, then, that most of the chromosome A's detected could be distinguished as to their arm types and arm combinations (Table 17). Forty out of the fifty-five chromosome types had the arm combinations of R–L, R–RL and RL–L and may be taken as one chromosome type, respectively. Each of the four chromosome types with an identical pattern in both arms (**101–101, 207–207, 208–208** and **301–301**) may naturally be regarded as one chromosome type. While each of the remaining eleven types had a pair of arms both belonging neither R nor L (cf. the asterisks in Table 17), and so might be taken as two different types (cf. the foot note to Table 17). The arm types of chromosome A's found in the populations investigated (Table 1) are re-listed in Table 18 making the distinctions of R, L and RL as above. The chromosome types less frequent than five per cent in each population are omitted from this Table because their frequencies were not only rare but also fluctuated widely even in neighboring populations. That is, they were rather fortuitous factors not characterizing the local distributions of this chromosome (Table 1). The local distributions thus represented coincided well with that distribution detected in respect to the arms of other chromosomes (cf. Tables 2–5 and the descriptions in the following). Two types of R's (**203** and **204**) and L's (**101, 205**) co-existed in **Tn** and either one of the two was found fixed in almost all the populations in northern Honshu and southern Hokkaido. One type of R (**201**) was seen fixed in northern Hokkaido populations. Its frequency declined towards middle Hokkaido where **101** took the place of it. It became fixed in **Ni** and **Tm** respectively. Three types of L's (**101, 103** and **207**) co-existed in **Sg.** The former two were found in **Np** and **My** together with another L (**213** in **Np** and **209** in **My**), and one or both of the latter two (**103** and **207**) were fixed or co-existed in northern Hokkaido populations and in **Tm.** Three types of R's (**201, 203** and **204**) and L's (**207, 208** and **306**) were the popular ones among Hidaka and eastern Hokkaido populations. Two or all of them co-existed in all these populations together with other several types of R's and L's which were less popular than the former.

All these findings gave the impression that the arm distinctions, though they were somewhat arbitrary, were reliable ones. It was tried, in the first step of the population analyses, to adopt this method of distinction as the first approximation until other, better methods are found.

The local difference in the distribution of various arm types of chromosome A was more or less modified and complicated, as in the case of chromosomes B and E (cf. Tables 2, 5 and Appendix Tables 2, 5), when the arm combinations were taken account. The modifications due to the combinations were slight in the populations of northern Honshu, southern, middle and northern Hokkaido, because of the smaller number of arm types included there and of the much restricted combinations of arms detected (cf. Table 18 and Appendix

Table 18. Distribution of arm types R and L of chromosome A in natural populations of *T. kamtschaticum*.

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Srr (S)	Srr (N)	Ty	Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
Type Number of R																											
104	—	—			—	-	—	-	—	—	—	—	-	—	—	—	-	-	-		-	—	—	—	—	—	
201	—	-	—	-	—	—	-	-	-	—	—	-	—	-		-	+	—	—	—	-	-	+	-	-	-	-
203	—	+	+	—	-	—	-	-	-	-	-	-	-	-	-	—	—	—		—	—	—	—	-		-	—
204	+	—	+	-	-	-	-	—	-	—	-	-	-	-	—	—	-	—	-	-		—		-		-	
101*	-	—	-	—	—	-	—		—	-	—	—	-	—	-	-	—	-	-	-		—	-	-	-	—	—
202*	-	—	-		-	—	-	-	-	—	-	—	—	-	—	+		—	-	—	—	-	—	-	-		—
207*	—	-	—	-	-	-	—		-	-	-	-	—	—	-	—	—	—	-	-		-	-			—	
208*	-	—	-		—	-	—		—	—	—	—	-	-	-	-	—			—		—	—	—	-		-
301*	-	-	—		-	-	—	—	-	—	-	—		-	—	—	-	-	-		-			—		-	
Type Number of L																											
101*	—	+	+	—	—	—	-	-	—	—	—	—	—	-	-	—	-	—	—	—		—	-	—	-	-	-
103*	-	—	-	—	-	—	—	-	-	-	-	—	—		—	—	—	-	-	-		-	—	-	-	-	-
202*	-	-	—	-	—		—	—	-		-	—	—	-	-	-	—	—	-	-	-	-	—	-		-	
207*	—	-	—		-		—	-	-	-	-	-	-	—	-	—	-	-	-		-	—	-	—		—	
208*		—		-	—	—	—	-	-	-	-	-	-	-	—	-	—	-	-	-	-	-			-	—	—
301*	—	-	-	—	-	-	-	-	—	-	-		-		-	—		—	-	—		—			-	-	-
205	+		+	-	-	-	—	-	—	-	—	-	-	-	-	—				—	-		—	-	—		
206		—					—	—			—	—	+	-	-	-	-	-	-	—		—			-	-	-
209	-	-		—			-	—	—	—	-	-	—	—		—	—	—	-	-	-			-		-	-
211	—	-	—			-	—	-	+	-	—	-			-	—		-		-		-					-
212	—	-	—	-	—	-	—	—		—	—	—		-	—	-	-	-		—		—		—			-
213		-		-		—	-		-	-	-	-	—	—	—	-		—	-	—	-	—	-	-	-		—
302							-	-		—	—	-	-		—	—		-	—	-		-	-				
306	-	-	-		-	-	—	—	-	+	+	+	-	+	+	+	-			—	—		—		—	-	-
307	—	—		-		-	-		—	-	—	—	—	—			-		-	—			—	-		-	—
310	—						-				-			-	-	-		-	-	-	-		-		-		-
202–308**		-	—	-	-	-	—	-	+		-	-	-	-	-	—		—	—	—			—		-	—	—

* The RL's which combine with L's were treated in this Table as R's, and those combining with R's were as L's.
** One chromosome type was impossible to distinguish R or L.

Table 1). They were somewhat conspicuous in Hidaka and eastern Hokkaido due to the preservation of a larger number of arm types and also to the various arm combinations. It may, however, be noted here that, even in such populations with numerous members, which may be regarded as panmictic (Narise '56), the combinations of arms were not free but were much restricted except in one population, **Srr**(**S**), in which all the possible arm combinations were found to exist (cf. Appendix Table 1). Accordingly it may be concluded that the recombinations of arms at kinetochore regions play a part in the production of chromosomal changes in natural populations of this plant subordinate to other mutational changes, to which attention will be paid in the following.

Mechanism of chromosomal mutation as revealed by the comparison of the differential patterns in chromosomes

As described above, recombinations of chromosome arms with different differential patterns seem to occur sometimes. New type chromosomes thus formed contribute to some extent to the chromosomal variations in natural populations of *T. kamtschaticum*. The variation due to recombinations must of course be preceded by structural changes of individual arms.

All the detected variations in chromosomes C and D were confined to their long arms. Accordingly, the variations of differential patterns in these arms may be attributable to paracentric structural changes in the long arms. All the types found in these arms were arranged in linear order based on their mutual morphological resemblance (Fig. 2, C–L and D–L). They are generally changeable from one to any of the neighbors due to minute structural changes such as inversions, duplications and deficiencies of small chromosome segments (Haga & Kurabayashi '54). Structural changes once produced may be enlarged by meiotic crossing-overs between homologous pairs with heteromorphic differential patterns.* These assumptions about the mechanism of structural changes in chromosomes were substantiated by comparisons of chromosome types found in a given population investigated.

Four types of chromosome C (**6, 10, 11** and **12**) were found in **Kk**. They may be mutually changeable from one another through any one of the mutational processes just described (cf. Table 2, C–L). Three predominant types of chromosome C (**1, 3** and **13**) found in eastern Hokkaido populations seem to be in the same mutual relation as the above four. Five types of chromosome C (**1, 2, 3, 5** and **6**) were found to occur frequently among Hidaka populations (**Sz** and **Sm**). These types revealed somewhat wider range of variation in their differential patterns than that of the former two groups of types. This is, perhaps, because of the co-existence of the two conspicuously different types, **3** and **6**. The former was one of the predominant types in the eastern Hokkaido populations and the latter was found fixed in almost all the populations

* Observations of meiotic chromosome behavior carried out with the PMCs at first metaphase of *T. kamtschaticum* indicated that the structural changes such as those concerning the variations in differential patterns did not greatly interfere with the pairing of homologous chromosomes.

in northern Honshu and in southern and northern Hokkaido. It seems plausible that the two types have been kept in co-existence at the balance of migration pressures, one from the east and another from the west (cf. Fig. 1 and Table 19). Meiotic recombinations in individuals heterozygous for such a conspicuously heteromorphic chromosome pair may result in wider range of phylogenetical variations in the patterns of differential chromosome segments than those in individuals with chromosome pairs which are slightly heteromorphic.

Total five types of chromosome D were found in **Np, Sg** and **My** (**1, 3, 9, 15** and **16**), and eight in **Sz** and **Sm** (**1, 2, 3, 4, 5, 6, 7,** and 8). The former populations are to be found between the northern and southern Hokkaido populations, in which type **1** and **3** predominated respectively over others. Types **1, 3** and **5** were respectively the most frequent ones in southern, northern and eastern Hokkaido populations (cf. Fig. 1 and Table 4). These findings indicate that the type found in the two groups of populations (**Np, Sg, My** ; and **Sz, Sm**) are those derivable from the types found in their neighboring populations due to meiotic recombinations between heteromorphic chromosome paris introduced there as a result of migrations. Most of the types of chromosome D found in eastern Hokkaido populations may be derivable from type **5,** which was the predominant one in these populations, by a few steps of chromosomal mutations such as minute inversions, duplications and deficiencies.

The variations in differential patterns of chromosomes C and D are those derivable from structural changes in the long arms not extending over the kinetochore. This may also be true in the majority of the cases of chromosomes B and E, because their long arms are generally fixed to one type, respectively (Tables 2 and 5). Their short arms revealed variation in differential patterns. Though the number of different types was smaller and the patterns of individual types were less continuous than those in chromosomes C and D, each of the types may also be derivable from others stepwise through minute structural changes.

Five and six types of short and long arms of chromosome B respectively were found in middle Hokkaido populations (**Sr, Np, Sg** and **My**). This is the only district in which a large number of types of these two arms co-exist with comparatively high frequencies. Evidence for pericentric structural changes, however, was found only once in one of these populations, viz., in **Sr** (cf. the description above and Table 8).

The two arms of chromosome A revealed the largest variation in differential patterns among the arms of the chromosome of this plant. The number of arm types, after making the distinction of R and L, is fourteen to seventeen in R and eighteen to twenty-one in L (Table 17). The variation in the patterns is so continuous that the minute structural changes due to the above noted mechanisms satisfactorily explain the production of the changes. This continuity makes it rather difficult to examine the relative importance of each of the mutational processes responsible.

The chromosomal variations detected in natural populations of *T. kamtschaticum* are, as described above, so large in scale and so great in number that they could hardly have been expected from the results of the direct cytological

observations hitherto carried out with meiotic and mitotic cells of this plant. This is by no means strange because the latter represent unsystematic haphazard examintations of accidental aberrations fortunately detected under the microscope, while the former are definitely the survivors accumulated over a long time. They are those which have escaped from random genetic drift and have endured continuous selection pressure. Gross chromosomal changes easily detectable under the microscope are deleterious in this plant as they usually are in other organisms. Only minute structural changes may be allowed to survive and become accumulated in natural populations. The inertness or the polygenic character of the differential segments (Darlington '47, Darlington & La Cour '41) may expand the latitude of their structural variability.

The variability of the individual chormosome arms generally corresponds with the quantity or the length of the differential segments to which the variations under investigation are confined. Thus the two arms of chromosome A and the long arms of chromosomes C and D revealed a wider range of variation than the arms of B and E. The variability of individual arms in a given population, however, does not always occur in the same way. Such may be the result of differential selection and mutation which the arms have experienced there. The predominance of D **5** in eastern Hokkaido populations may be the result of effective selection for this type in these populations, and the wide variations of C and D in **Sz** and **Sm** may be attributable to their increased mutation rates, very probably due to the conspicuous heteromorphism of the chromosome types introduced there by way of migration from neighboring districts (cf. Fig. 1 and Tables 3 and 4).

These assumptions are made without any experimental evidence or phylogenetical test. However, they are supported invariably from the mode of local distributions of chromosome types as described in the following (cf. Tables 2-5, 18 and 19).

Isolation and geographical races of *T. kamtschaticum*

The chromosomal variations in natural populations of *T. kamtschaticum*, as described above, indicate clearly the local differentiation of this plant. Here it is possible to distinguish four population groups geographically as follows: **North,** which includes populations **Fd, Kk, Rf, Od, Kb, Sn, Ke** and **On**; **Middle,** including **Np, Sg, My, Tm** and **Ni**; **East,** including **Sz, Sm, Srr(S), Srr(N), Ty, Ms, Ak** and **Ot**; and **South,** including **Ks, In, Tn, Hg, Sr** and **Sd.** After making this grouping, the chromosomal composition of the populations within and between each group was compared with arm types which had frequency of more than five per cent in each population (Table 19).

After the comparison of **Srr(S)** and **Srr(N)**, an astonishing agreement in the chromosome types detected in these two populations was found (Tables 1–5, 18, 19 and Appendix Tables 1–5). Only a few chromosome types of rare frequency were found in one of them and not in the other. The difference in the frequency of each type included in the two populations may be no more than the result of random fluctuation which occur because their members are

not entirely panmictic (cf. Narise '56). The difference in chromosomal composition between **Ty** and **Ms** was somewhat larger than that between the former two populations. It may largely be attributable to the effect of random genetic drift which operates slightly in **Ty** but does in some moderate degree in **Ms**, and to that of sampling error, the number of plants analysed in **Ms** being far smaller than that in **Ty**.

The neighboring populations examined in **East** were all in similar mutual relation to that just described. The difference between each neighboring two was determined due to isolation by distance and to their effective size. Thus there could be found four subgroups in the group **East**: **SZM**=**Sz** and **Sm**, **SRR**=**Srr**(**S**) and **Srr**(**N**), **TMS**=**Ty** and **Ms**, and **AO**=**Ak** and **Ot**. Each of the subgroups was so grouped because of the intimate resemblance in chromosomal composition (Table 19). A population, **Hr**, which is located between **SRR** and **TMS**, was examined; it was found to have just the intermediate chromosomal constitution between the two population pairs (Appendix Table 1-5).

Populations in **South** are, today, far more isolated than those in **East**, no continuity in distribution being discernible in the former. On the other hand, their resemblance in chromosomal composition was, as described above (cf. Tables 1–5, 18 and 19), not a mere chance coincidence. Based on the resemblance they were grouped into subgroups: **KIT**=**Ks**, **In** and **Tn**; and **HSS**=**Hg**, **Sr** and **Sd**. The difference in chromosomal composition between each population pair of these two subgroups was far larger than that between any pairs in **East** as was expected from the degree of continuity in distribution and the population size. These two subgroups yet include enough numerous common types, which characterize them to be distinguished from the populations of other groups than **South**.

Five populations along or near the Ishikari Depression were investigated in **Middle** (Fig. 1). Three populations (**Np**, **Sg**, **My**) which are located in the northern part of this Depression were so similar in chromosomal composition that they were grouped into one subgroup, **NSM**. The remaining two, **Tm** and **Ni**, found in the southern part of the Depression, were so different not only from each other but also from any other neighboring populations that they were left out of subgrouping.

All the populations investigated in **North** had many type in common, some of which were characteristic ones there. They were subgrouped, on the basis of resemblance in chromosomal composition into two: **RKO**=**Rf**, **Od**, **Kb**, **Su**, **Ke** and **On**; and **FK**=**Fd** and **Kk**.

The right arm of chromosome A was fixed to **201** in **North**, while this arm was represented in **South** by two types, **203** and **204**. These three types coexisted in **East**. In addition other types of the right arm (**104**, **207**, **208**, **301**), inherent to **East** were also found in some of the populations there. Each one of the three different types of the left arm of chromosome A, **103**, **205** and **306**, respectively was the most frequent and common type in **North**, **South** and **East**.

Two types of chromosome C (**6**, **10**) predominated in **North**. One of them, type **6**, was found nearly fixed in **South**. While in **East**, another different type, **3**, was the most frequent one in each population. Numerous types other than

this were found there. They were inherent ones to **East,** except type **6** found restricted to subgroup **SZM. North** populations had only one type of chromosome D **1,** in fixed condition, while **3** and **5** were the most frequent and inherent ones in **South** and **East** respectively.

Five types of the short arm of chromosome B (**101, 102, 201, 202, 302**) were found to occur in **South.** Two of them, **102** and **202,** were the characteristic ones there. One of the latter two, **102,** was found however, restrictedly in one population, **Sr,** and so cannot be the representative of this group. Most of the populations in **North** and **East** had two predominant types, **201** and **301,** in common. One characteristic type, **104,** was found in **North.** However, it was included only in one subgroup, **FK.** Accordingly this type cannot be regarded as the common element of **North**.

Two types of the short arm of chromosome E co-existed in **North.** One of them, **302,** which was found in both of the two subgroups in **North,** was the inherent type for this group. Another one, **204,** was found also in **East,** together with three other types (**203, 202** and **101**) frequently. One of the latter three, **203,** was the one inherent to **East.** Only one type, **201,** was included in common in both of the two subgroups in **South.** This type was found only rarely in a fraction of the subgroups of **East.** So that it may be allowable to regard it as the representative of **South.**

The long arms of chromosomes B and E respectively were fixed to one and the same type in the majority of the populations investigated. Accordingly, little differentiation among the three major groups was detectable with respect to these arms.

The three groups, **North, South** and **East,** had, as just described (cf. Table 19), at least one type characterizing each of them, with respect to each of the five chromosome arms (A–R, A–L, C–L, D–L, E–S) out of the eight arms, which revealed structural variations in differential segments (cf. Fig. 2). Two of the remaining three arms were those revealing the least (E–L) and the next to the least (B–L) variation in their differential patterns (Fig. 2). So, it is no wonder that they show little local differentiation with respect to the patterns of their differential segments. The larger the number of arm types detected in chromosome arms was the more the local differentiation in chromosomal composition became conspicuous. Actually, no type with high frequency was found in common with respect to A–L and D–L, which are the most and next to the most variable ones respectively, in any two of the three groups. As the variability of arm patterns decreased, the number of types commonly found in two or all of the groups increased, and that of those inherent to each decreased. Such may be the indication of the influence of mutation frequency upon the production of populational variations. Increased mutation facilitates the differentiation of natural populations. There, the mutation pressure acts coping with selection, and random variations in the differential patterns may be sorted out with respect to their adaptiveness. Increase in chromosomal variability may increase the chances for different chromosome types to be adapted in different population groups which exist respectively in different environmental conditions. Poor variability, on the contrary, may sometimes result in some different adaptation

without difference in chromosome structure.

The environmental conditions in the districts where **North, East** and **South** are distributed fairly differ from each other. Further the population dynamics operating among the populations in each group differ much, as described below, both in quality and in quantity.

In **East,** each population possesses such a large number of effective members and is isolated from others so little that it is under the influence of active mutation and selection pressure. Actually, all the populations investigated in **East** were quite heterogeneous, having a large number of chromosome types. The kind and frequency of the types found in each of the populations were roughly coincident. In **North** and **South,** on the contrary, most of the populations were far more isolated from each other and far smaller in size than those in **East.** It seems that, in some of the populations in the latter two groups, selection and mutation have scarcely been effective since complete or nearly complete fixation of chromosome types in each pair occurred. In spite of such decrease in variability, predominant types common in all the subgroups in **North** and **South** respectively were recognized (Table 19). This genetical continuity among these subgroups with respect to the arm types seems to indicate the influence of selection pressure. This agency, however, can hardly be effective in operation at present, because, as just described, genetic variability has been doomed to stand still among the individual populations found there. Accordingly, the continuity revealed in **North** and **South** may not be contemporary but may be historical. The geological history of these two districts indicates that there had existed conditions to allow expansion of population size sufficient to admit adaptive evolution in some Ice Ages (Yabe '29, and also see the following descriptions of this paper).

Selection and random genetic drift are the mutually exclusive agencies of evolution. The latter takes the place of the former in the decrease in effective population size. However, if a population size decreases gradually, or fluctuates up and down around a certain number, these two factors may be acting cooperatively so as to decrease genetic variability of the population more or less directively towards adaptive fixation (Kerr & Wright '54, Wright & Kerr '54, Hiraizumi, in press and Kurabayashi et al. '56).

The populations of **East** are distributed in Hidaka and eastern Hokkaido where few isolation barriers against this plant are found. All the populations of **East** are large and generally continuous at present. The distribution is sometimes interrupted by man's cultivation. The influence of such cultivation upon the genetic composition of the populations, however, may be negligibly small because it has been begun within the past few tens of years. The extension of glaciers along the Hidaka mountain range, marine invasions during the last Ice- and Inter-Ice Ages and the recent volcanic activities (Yabe '29, Minato et al. '53, Minato '54, etc.) might have altered the geographical and ecological conditions of this district. They have, however, failed to leave any marked traces in the present populations concerning, at least, the distribution of chromosome patterns. Only slight discontinuity in respect to the distribution of some chromosome types is to be found in some of the subgroups.

There were four arm types (**301** of A–R, **301** of A–L, **206** of A–L and **302** of B–S) restrictedly found in **AO** with fairly high frequencies. They were absent or rare in **TMS** and other subgroups in **East.** Type **208** of A–L was one of the popular ones in **TMS, SRR** and **SZM,** while it was rare in **AO.** The area between the subgroups AO and TMS, though it was wider than others separating other neighboring subgroups in **East,** is continuously habitated by this plant at present. There seems, however, to have existed in ancient times conditions which separated the subgroups AO and TMS, because this area is widely covered with volcanic deposites of various origins. The cliff at the southern foot of Mt. Apoi (cf. Fig. 1) seems to have acted as a barrier between **SRR** and **SZM,** because elements characteristic to **North** and **South** are rarely found to pass eastward across this barrier.* It may be probable, however, that the discontinuities just described would be replaced by some gradational changes when sufficient numerous populations living between the subgroups are analysed as was actually found to be the case between the subgroups **TMS** and **SRR** (cf. **Hr** population in Appendix Tables 1–5). In general, the populations in **East** were large in size and nearly continuous with each other in the past as they are at present. The main factors guiding their evolutionary changes have been, to speak briefly, mutation and natural selection.

The populations in **South** were divided into two subgroups, **HSS** and **KIT.** They were genetically the most discontinuous ones among the subgroups established in each group. The individual populations in each of the two subgroups were also discontinuous. Such may be the consequence of random genetic drift among these populations, which, at present, distribute separately and are more or less reduced in size. In spite of such discontinuity, it becomes conceivable from the comparative examination of their chromosomal composition that they were in reproductive connection with each other sometimes (and not always) in the past.** That is, they have 'historical' continuity. The present continuity is the direct sequence of exchanges of genetic materials among populations now reproductively continuous. The historical continuity is the residue of past panmixia among populations continuous in the past. If selection had not been effective to make certain adaptive genotypes sufficiently frequent in each of the populations while they were panmictic, or if random genetic drift, which had begun since the breakdown of the reproductive continuity, had been rapid and severe, the historical continuity would have failed to be preserved. The continuity would also have been rendered obscure in the process of re-expansion of population size after removal of conditions which induced the breakdown of continuity.

The difference in the degree of continuity as revealed by comparisons of

* The influence of migration from the west into **SZM** was inferred from the presences of the following arm types: **204** of A–R, **6** of C–L and **1** of D–L, which were frequent ones in **North** or **South** but rare or absent in the subgroups of **East** other than **SZM**.

** The evidence for the past continuity may be obtained after the comparison of the chromosomal compositions of **Tn** and **Sr** with which the populations of subgroup **KIT** and **HSS** respectively seem to have kept in intimate connection (cf. Tables 2–5, 18, 19 and the following descriptions).

chromosomal composition between and within the subgroups in **South** may be understood as the synthetic results of cooperation among the just mentioned three factors, selection, drift and re-adaptation.

The Tsugaru Strait (Fig. 1), which now isolates the two subgroups, **HSS** and **KIT,** has been the site of a land bridge for some time during the last million of years due to the lowering of sea level in periods of glaciation (Yabe '29, etc.). *T. kamtschaticum* had then made radiation southward extending the present limit of distribution into the area of the present main island of Japan. The rising of the sea level, the warmer climate and the volcanic activities during, especially, the last hundreds of thousands of years, and more recently man's cultivation, which have been conspicuous in the main island of Japan, have all acted in cooperation to separate the two subgroups and to reduce the size of the individual populations which have become more and more isolated since then.

The historical continuity of the two subgroups is inferable most concretely from the fact that they jointy own the two arm types, **205** of A–L and **3** of D–L. The former is found also in some populations of **East.** However, the frequency of this type in these populations is so small except in **Ms** (Tables 1 and 19) that it may be taken as one of the characteristic types in **South.** It seems, however, that this assumption is not correct because **In,** one of the populations in subgroup **KIT,** does not include this type but had **101** of A–L in fixed condition (Tables 1 and 19). This may further be understood as correct after the following considerations.

That is, the two types, **205** and **101,** co-exist in **Tn,** the neighboring population of **In** in **KIT.** There must have been, during the Ice Ages, periods when the populations of this subgroup had expanded to have a common gene pool. Since the onset of the Post Ice Age, the contionuity was broken. Then the random genetic drift progressed in various degrees in each population in proportion with the reduction of its size. This drift has not yet become very advanced in **Tn** and the historical continuity has escaped from being extinguished due to the existence of this population. For these reasons, it may be concluded that the absence of **205** in **In** is not an argument against the continuity of this population with others in **South.**

The same thing may be said about the absence of **3** of D–L in **Sd** of **HSS.** This type is the inherent one to **South** and is found in no population in other group than this. The historical continuity with respect to this type in **HSS** is established by the presence of **Sr,** as it is done by **Tn** in the above case (cf. Tables 1–5, 18 and 19). Thus the historical continuity between **KIT** and **HSS** becomes clear by the mediation of the two chromosome types, **205** of A–L and **3** of D–L, and of the two populations, **Tn** and **Sr.** It is a notable fact, though the historical continuity is established evidently as such, that the random nature of genetic drift is revealed more conspicuously in **South,** which occupies the southern limit of distribution of this plant in Japan, than in any other groups.

The genetic discontinuity among the three subgroups in **North** was as small as that among those in **East** (Table 19). The genetic continuity in the former

is preserved jumping over the present geographical barriers which separate some of the populations in **North.** The populations in Rishiri Island, for example, have a chromosome composition which differs little from that found among the populations in the northern district of the main island of Hokkaido. Some difference due, perhaps, to random genetic drift was seen in the genetic composition between the subgroup in Rebun Island (**FK**) and others in **North.** It was too slight, however, to substantiate any idea of their genetic discontinuity.

The populations in **North** have sometimes shown reproductive continuousness in recent Ice Ages because this district might have been the main route of migration of this plant from north to south, or *vice versa,* along the Japanese Islands. It would be possible that an adaptive peak happened to be established during the migration. The predominance of coniferous woods in the northern part of the main island of Hokkaido would have represented the conditions favorable for gradual and fluctuating change in effective size of the populations of this plant. In such circumstances, they have been obliged to stick to the once established adaptive peak. Thus the historical continuity has been maintained at the expense of genetic variability.

The genetic continuity in each of the three major groups was substantiated in contrast to the discontinuity among them. The discontinuity was marked along the Ishikari Depression (Fig. 1), where the three groups meet. This Depression has been brought above the sea level by the latest Quarternary deposits of river sediments and volcanic products during the last few thousands of years. The slight lowering of sea level since the end of the 'climatic optimum' in post glaciation has promoted the connection of this land bridge (Nagao '40, Minato et al. '53, Minato '54, Yabe '29, etc.). This region, thus, has recently been an effective geographical barrier for only a few thousands of years during the last maximum marine invasion. After this invasion, migrations of *T. kamtschaticum* took place with various frequencies and directions and left traces in the chromosomal compositions of some populations distributed along the Depression.

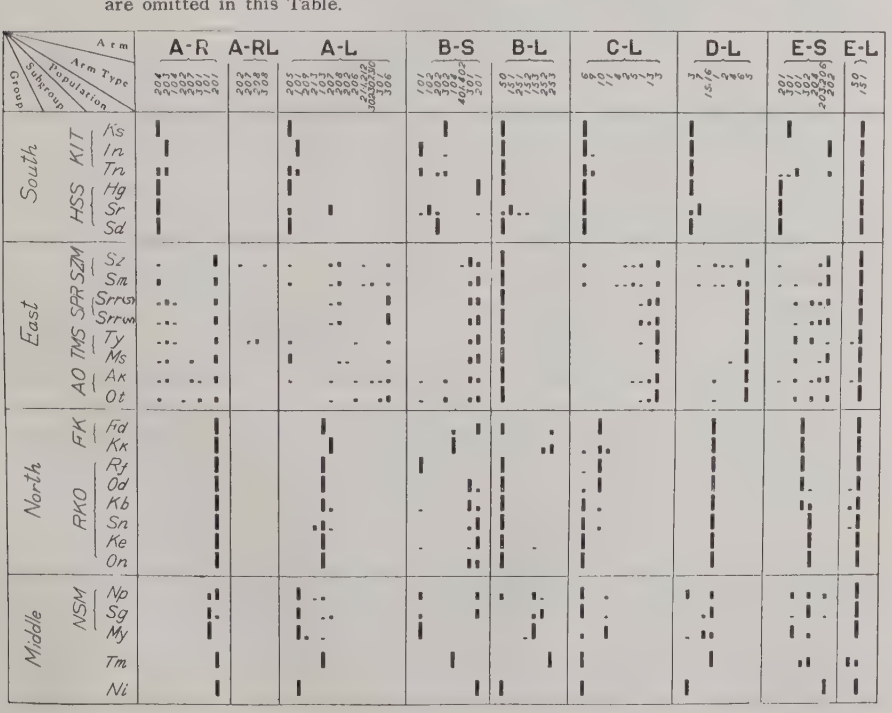
Several populations were chosen for investigation in the northern part of this Depression (Table 19). They were bestowed with abundance of northern elements in addition to those inherent to them. Such a situation was reasonably expectable from the geographical and ecological conditions of the regions where the populations are found. That is, the deposits of the rivers Ishikari and Toyohira repeatedly afforded suitable conditions for an expansion in the size of these populations in connection with the northern group. The expansions allowed some latitude for trial and error to evolve a new chromosome constitution peculiar to this population group (subgroup **NSM**).

The southern part of the Depression is covered with deposits from recent volcanic activities. Only limited areas being healed from such cover allow the habitation of this plant. The populations found there were small in size and isolated widely from each other.

Tm was found to be small in size and high in homogeneity (Appendix Tables 1–5 and Hiraizumi '56). It included an abundance of northern elements some

of which were the identical ones inherent to the subgroup **FK** (Table 19 and Fig. 1). Its geographical position never implied any recent-day connection between it and subgroup **FK** beyond the intervening populations (Fig. 1). So the continuity, which is too intimate to be by chance coincidence, might be an indication of the historical connection which had been maintained as the result of the random genetic drift in combination with natural selection which had been so effective as to enable the maintenance of the adaptive peak throughout the gradual and fluctuating decrease in the size of **Tm.** **Ni** is composed mostly of southern elements (Table 19). However, some contribution to this from

Table 19. Local distribution of arm types. The length of the rods drown in each column represent the relative frequency of the corresponding arm types in the corresponding populations. Some arm types (**211** and **212**; and **302, 307** and **310** of A–L: **401** and **402** of B–S: **15** and **16** of D–L: and **203** and **206** of E–S) are grouped together in this table because the grouping does not alter the general mode of local distribution of arm types. The distinctions of right (R) and left (L) arm of chromosome A are made after the manner employed in Tables 12–16 and 18. Types impossible to decide whether R or L are listed in this Table as RL. Chromosome types which do not attain five per cent (cf. Appendix Tables 1–5) of the total chromosomes analysed in each population are omitted in this Table.



the subgroup **NSM** was inferrable from the type of one of the chromosome pairs (Chromosome A, cf. Table 19).

Migration from the west towards the east across the Depression also took place, as evidenced by the fact that a few southern elements were found among the populations of Hidaka (subgroup **SZM**).

Migrations across this Depression, however, are so small in scale and so restricted in range that the three population groups, **North, East** and **South,** were kept nearly isolated. The plants in each group differ from those in the others not only in their chromosomal composition but also in some of their external morphological features (Suzuki '57) and physiological characters. Thus the three groups are respectively on the way of racial differentiation. It seems that there are some signs of chromosomal sterility introduced by migrations of heteromorphic chromosomes from one group to another (Haga '52). Development of such mechanism may assist this differentiation.

The present investigation were carried out under the guidance of Prof. Haga of Kyushu University, and of Prof. Matsuura and Prof. Minato of Hokkaido University. The writer wishes to express here his cordial thanks. Thanks are also due to the researchers of our institute. The population analyses have been carried on with their cooperation.

Conclusion and Summary

The structural changes in the differential segments of chromosomes of *Trillium kamtschaticum* Pall. (2n=10) as revealed under low temperature condition were investigated with plants obtained from twenty-nine natural populations.

It was found that the patterns of differential segments, in which the structural changes under investigation are detected, revealed somewhat discontinuous variations allowing one to distinguish the individual patterns found in each chromosome arm. The number of different patterns of the segments of each chromosome arm attained 29, 11, 10, 7, 15, 16 and 4 as to the two arms of chromosome A, as to the short arms of chromosomes B and E and as to the long arms of chromosomes B, C, D and E, respectively (Fig. 2).

These different patterns or 'types' of each arm may be derived from ancestral types or be differentiated from each other due to chromosomal mutations such as inversions, deficiencies and duplications. Little could be learned about the mechanism and frequency of the mutation. It was an observed fact, however, that there were certain particular types, not so numerous in number, which were frequent and common in a certain group of populations. These types may be regarded as the representatives of the populations where the types have been kept and propagated as the members of their one gene pool. Such a fact may indicate that the individual types, though they are changeable, are fairly stable transmitted unchangeably through generations. That is, the types of each chromosome arm may be treated as a series of multiple alleles with mutation rates not unexpectedly high.

There were three chromosome pairs, A, B and E, with detectable structural

changes in the differential segments of their two arms (Fig. 2). Through combinations of different arm types of the short and long or right and left arm, different chromosome types are born. The combinations, however, were shown to occur so restrictedly in chromosome B and E that they contribute only a little to the chromosomal variation among the natural populations investigated (Tables 6-10). Matters were somewhat complicated in the case of chromosome A, because it is isobrachial and its right and left arms could not be distinguished from each other by way of simple comparisons of the patterns in the differential segments. It became approximately possible, however, through examinations of the combinations of arm types, to distinguish the two arms from each other. The arm combination, investigated after this distinction of the right and left, was not free but occurred only restrictedly. The remaining chromosome pairs, C and D, revealed structural variations only in the differential segment of the long arms.

The general aspects of the chromosomal composition in each of the natural populations investigated were found little altered, owing to the limited arm combinations among the three chromosomes, A, B and E, whether the chromosomal composition was represented with chromosome types (Appendix Tables 1–5) or with arm types (Tables 2–5, 18). Accordingly, in the present paper, the population analyses were made on the basis of the arm type distributions.

As indicated in Table 19, the number, kind and frequency of arm types differed within and between populations. The differences among the populations were by no means random. Some populations had several chromosome types in common which were not found among other populations. Some were homogeneous and others were heterogenous. It was noted from this Table that populations which possess some common chracteristics in respect to the chromosomal compositions were generally those obtained from one and the same geographical district (Table 19 and Fig. 1). Thus it is possible to distinguish three geographical population groups, **North, East** and **South,** which respectively have certain different characterstics in chromosomal composition as well as in geographical position.

North occupies the northern part of Hokkaido. That group has five arm types which attain predominant frequencies in almost all the populations there. Two of the five arm types were inherent ones found scarcely among the populations of any other district. Each chromosome arm found in **North** was represented, in the majority of cases, by a small number of types, generally one or two, which were, as a rule, common in more than half of the populations in **North.** Such inter- and intra-populational high homogenetity was assumed to have been attained through persistence of the adaptive peak which had been attained in **North.**

East includes populations distributed in Hidaka and eastern Hokkaido. Three arm types were found as the representatives of **East.** All the populations here were quite heterogeneous in contrast to those in **North.** The majority of chromosome arms in each population were represented by numerous types including the three typical representatives. The populations in **East** are all large in size and little isolated from each other. It seems that they are as

a large group on the way of adaptive evolution.

South includes populations in northern Honshu and southern Hokkaido. Here the distribution of. *T. kamtschaticum* is much restricted and discontinuous. The homogeneity of each of the populations was high. Most of the chromosome arms were fixed to one type or were represented by only a few types. The fixations among the individual populations were, as compared with those in **North,** somewhat random especially among those in the subgroup **KIT,** which is found at the southernmost end of the distribution area of this plant in Japan. It was concluded that the high homogeneity was brought about here mainly by way of random genetic drift. Only one arm type was found to be the unique one generally found in **South.**

Another group of populations, **Middle,** was investigated in the Ishikari Depression. This is the region where the above described three groups meet (Fig. 1). In the northern part of this depression develops one group of populations (subgroup **NSM**) which suffer mostly the influence of migration from **North.** The southern part of this depression is populated very scarcely by this plant. Two populations, **Tm** and **Ni,** were investigated here. They were both small in size and high in homogeneity. The former had identical chromosomal composition with **FK** in **North. Ni** was conspicuously influenced from **South,** but include also a few elements of **North** and of the subgroup **NSM.** Migrations on a small scale were recognized as having occurred across this depression from **South** into **East.**

The existence of the three geographical population groups and their interrelationships along the Ishikari Depression where they meet, is an indication of the development of racial differentiation in *T. kamtschaticum.* The process of this differentiation was presumed to have been influenced not only by the eco-geographical conditions prevailing at present in northern Japan but also by those in ancient times, especially those during the late Pleistocene Epoch.

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Appendix Table 1. Type and frequency of chromosome

Polulation		Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Hz	Srr (S)
Arm-Type R - L	Chromosome Type**												
101-101	53												
101-103*	52												
101-207*	50												
101-208*	25												1
101-209*	51												
102-208	11			1									
102-306	37												1
103-205	12									1		1	
104-206	29												
104-207	41												
104-208	35												3
104-306	33												7
201-101	38							30					
201-103	1								8	3	1		
201-205	3									4	9	2	3
201-206	24											7	
201-207	18									11	2		
201-208	2									22	14		12
201-211	4									6	1		
201-213	54												
201-302	6										7		
201-303	44											1	
201-306	5									25	19	2	24
201-310	26												
202-206	28												
202-308*	19									14	2		1
203-101	22		100	33									
203-202	55												
203-205	21			8									
203-207	31											1	3
203-208	39											3	2
203-302	8									1	3		
203-306	30											1	20
204-205	13	100		46	(11)	44	(7)				6	1	
204-207	14					56				5	5		
204-208	16									5	12		
204-211	43												
204-212	15										9	1	1
204-306	36												6
204-307	9									3	5		
204-401	46												
207-207	32												2
207-208*	17										2		3
207-306	10										1		
207-310	47												
208-208	23												
208-209*	42												
208-305*	7										2		1
208-308*	45												
209-402*	48												
210-309*	40												
301-301	20												
301-303	27												
301-306	49												
304-306	34												
Total		100	100	88	(11)	100	(7)	30	8	100	100	20	90

* The eleven chromosome types marked with one asterisk respectively are those in

** The number of chromosome type written in the second column were given, without dis-

A in natural populations of *T. kamtschaticum*.

Srr (N)	Hr	Ty	Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
.	—	—	—	—	—	—	—	—	—	—	—	—	. .	14	29	15
.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
.	—	.	—	—	—	—	—	—	—	—	—	—	.	—	8	—
1	—	—	—	1	1	—	.	—	—	—	—	—	—	—	1	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
1	4	3	—	3	—	—	—	—	.	—	—	—	—	—	1	
—	1	—	—	1	4	—	—	—	—	—	—	—	—	—	—	—
.	—	—	—	—	.			—					.			
—	—	6	—	2	1	—	—	—	—	—	—	—	.	—	—	—
1	—	2	—	.	—	—	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—	—	—	.	—	—	—
6	1	—	—	1	—	—	—	—	—	—	—	—	.	—	—	—
	—	.			2	—	—	—	—	—	—	.	—	18	6	—
—	1	2	—	—	1	22	—	100	25	27	6	14	20	6	5	—
1	1	18	15	7	1	—	.			—		. .		.	.	
1	2	5	—	9	6	—	—	—	—	—	—	—	—	—	—	—
—	11	8	—	8	6	—	48	—	1	7	1		.	.		
5	—	6	1	2	—	—	—	—	—	—	—	—	.			
—	—	1	—	—	—	—	—	—	—	—	—	—	—	—		
.	—	—	—	—	—	—	—	—	—	—	3	—	—	2	—	—
—	—	4	1	—	1	—	—	—	—	—	—	—	—	—	—	—
—	—	3	—	—	—	—	—	—	—	—	—	.	—	—	—	—
33	13	11	—	4	9	—	—	—	—	—	—	—	—	—	.	
—	—	6	—	9	—	—	—	—	—	—	—	—	—	—		
—	—	1	—	2	11	—	—	—	—	—	.	—	—	—	—	—
—	1	4	2	2	2	—	—	—	—	—	—	—	—	—		.
	—	—	—	—	—	—	.	—	—	—	—	—	—	—		
—	—	—	2	1	—	—	—	—	—	—	—	—	—	—	—	—
2	1	5	5	2	—	—	—	—	—	—	—	—	—	—	—	—
4	1	7	—	4	—	—	.	.	.	.	.	.	.	.		
—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	
—	—	3	—	5	—	—	—	—	—	—	—	—	—	—		-
16	9	21	. .	2	4	—	—	—	—	—	—	—	—	—	—	
—	—	2	—	—	—	—	—	—	—	—	—		.	.		
—	—	1	—	3	—	.		.				—			.	
—	—	3	4	1	—	—	—	—	—	—	—	—	—	—		
—	—	3	—	—	—	—	—	—	—	—	—	—	. .	—	—	
2	—	5	—	3	—	.		.			—		. .		.	
5	—	5	—	—	16	—	—	—	—	—	—	—	—	—	—	—
—	—	1	—	2	2	—	.	—	—				.		.	
—	—	1	—	—	—	—	—	—	—	—	—	—	.		.	
—	2	2	—	5	2	—	—	—	—	—	—	—	—	—	.	
4	3	8	—	1	—	—	—	—	—		.		.			.
1	9	4	6	9	4	.	—		—	. .	.	.	.			—
—	1	—	—	—					.	.	. .	.				
—	1	10	—	—	1	—	—	—	—	—	—	—	—	—	. .	
—	—	1	—	—	—	—	—	—	—	—	—	—	—	—		
—	—	4	—	1	2	—	—	—	—	—	—	—	—	—		—
—	—	2	—	—	—		.			—	. .	.	.			—
—	1	—	—	—	—	—	—	—	—	—	—	—	—	—		
—	—	3	—	1	—	—	—	—	—	—	—	—	—	—		.
—	1	—	—	6	22	—	—	—	—	—	—	—	—	—		.
1	—	—	—	1	2	—	—	—	—	—	—	—	—	—	—	—
—	—	1	—	—	—	—	—	—	—	—	—		—	—		
1	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	
90	66	172	36	100	100	22	48	100	26	34	10	14	20	40	50	20

which it is impossible to distinguish their right and left arms (cf. Table 17).
tinction of right and left arms, after the order of their discovery (cf. Haga & Kurabayashi '54).

Appendix Table 2. Type and

Population		Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Hz	Srr (S)
Arm-Type R-L	Chromosome Type												
101—50	1	—	86	43									
101-152	9	—	—	—	—	9							
101-153	13	—	—										
102-151	6	—	—			61						—	—
102-251	11					1						—	—
104-252	15	—	—							—	—	—	—
104-253	16	—	—						8	—			
201—50	2				(11)	—		30	—	31	63	6	49
201-251	8					7		—	—	—	—		—
201-252	10	—	—	1		4		—	—	—			—
202—50	7	—	—	20	—	18	(7)				1		
204—50	17	—	—										
205-151	18											—	—
301—50	3									57	33	14	41
301-152	14	—											
302—50	12	100	14	24				—	—	1	1	—	—
401—50	4	—	—	—				—	—	6	2		
402—50	5	—	—					—	—	5	—		—
Total		100	100	88	(11)	100	(7)	30	8	100	100	20	90

Appendix Table 3. Type and

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Hz	Srr (S)	Srr (N)	Hr
Chromosome Type														
1	—	—	—	—	—	—			16	7	2	5	29	—
2	—	—	—	—	—	—			12	8	—	—	—	—
3	—	—	1	—	—		—	—	38	31	2	48	46	48
4	—	—	—	—	—				2	6	—	—	—	1
5	—	—	—	—	—	—	—		10	22			—	—
6	100	95	64	(11)	100	(7)	30	8	16	16	—			—
7	—	—	—	—	—	—			4	2	—	—	—	—
8	—	—	—	—	—				1	7	—	—	—	
9	—	5	23	—					1	1	—	—	—	—
10	—	—	—	—	—				—					—
11	—	—	—	—	—				—	—	—	—	—	—
12	—	—	—	—		—			—	—	—	—	—	—
13	—	—	—	—	—	—	—		—	—	16	35	15	16
14	—	—	—	—		—	—		—	—	—	—	—	1
15	—	—	—	—	—	—	—		—	—	—	2	—	—
Total	100	100	88	(11)	100	(7)	30	8	100	100	20	90	90	66

frequency of chromosome B.

Srr (N)	Hr	Ty	Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
		6	—	6	6	—	—	100	—	3	—	—	—	—		
		—	—	—	—	—	—	—	—	—	—	—	—	1	1	2
												1	—	20	16	18
		1														
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		
					—	7	19	—								
—	—	—	—	—	—	—	27	—								
52	25	100	23	29	35	15	2	—	5	9	8	11	9	15	1	
	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	11	5	—	—	—	—	—	—	—	—	—	—	—	3	31	
—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	3	2	—	—	—	—	—	—	—	—			
		1	—	—												
38	28	59	13	40	27	—	—	—	21	22	2	2	11	1	1	
	2	—	—	—	—	—	—	—	—	—	—	—				
—	—	—	—	22	29	—	—	—	—	—	—	—	—	—	—	
90	66	172	36	100	100	22	48	100	26	34	10	14	20	40	50	20

frequency of chromosome C.

Ty	Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
9	—	10	5	—	—	—	—	—	—	—	—	—	—	—
1	—	3	—	—	—	—	—	—	—	—	—	—	—	—
155	33	62	75	—	—	—	—	—	—	—	—	—	—	—
—	2	—	—	—	—	—	—	—	—	—	—	—	—	
1	1	5	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	—	—	9	7	4	27	8	14	20	33	45	6
			—	—	—	—	—	—	—	—	—	—	—	—
3	—	2	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	22	23	93	22	7	2	—	—	1	—	—
—	—	1	—	—	15	—	—	—	—	—	—	6	5	14
—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
2	—	16	20	—	—	—	—	—	—	—	—	—	—	—
—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
1	—	—												
172	36	100	100	22	48	100	26	34	10	14	20	40	50	20

Appendix Table 4. Type and

Population	Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Hz	Srr (S)	Srr (N)	Hr
Chromosome Type														
1	—	—	—	—	—	—	—	8	20	7	—	—	—	4
2	—	—	—	—	—	—	—	—	12	8	—	—	—	—
3	100	100	88	(11)	27	—	30	—	3	2	—	—	—	—
4	—	—	—	—	—	—	—	—	6	2	1	—	—	—
5	—	—	—	—	—	—	—	—	47	26	11	90	90	61
6	—	—	—	—	—	—	—	—	2	39	1	—	—	
7	—	—	—	—	73	(7)	—	—	9	14	1	—	—	—
8	—	—	—	—	—	—	—	—	1	2	—	—	—	
9	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—	1	—	—	—
13	—	—	—	—	—	—	—	—	—	—	2	—	—	—
14	—	—	—	—	—	—	—	—	—	—	3	—	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—	1
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	100	100	88	(11)	100	(7)	30	8	100	100	20	90	90	66

Appendix Table 5. Type and

Population		Ks	In	Tn	Hg	Sr	Sd	Ni	Tm	Sz	Sm	Hz	Srr (S)
Arm-Type R-L	Chromosome Type												
0—50	16	—	—	—	—	—	—	—	—	—	—	—	—
101—50	11	—	—	—	—	—	—	—	—	—	—	—	—
101–151	1	—	—	46	—	—	—	—	—	4	9	6	14
201–151	2	—	—	8	(11)	100	(7)	—	—	9	4	—	—
202–151	3	—	100	22	—	—	—	30	—	65	67	8	35
202–152	6	—	—	—	—	—	—	—	—	—	2	—	—
202–251	15	—	—	—	—	—	—	—	—	—	—	—	—
203—50	8	—	—	—	—	—	—	—	—	—	—	—	—
203–151	4	—	—	—	—	—	—	—	—	22	17	3	14
203–152	5	—	—	1	—	—	—	—	—	—	1	—	—
203–251	12	—	—	—	—	—	—	—	—	—	—	—	—
204—50	17	—	—	—	—	—	—	—	5	—	—	—	—
204–151	10	—	—	—	—	—	—	—	—	—	—	3	26
206—50	14	—	—	—	—	—	—	—	—	—	—	—	—
301–151	7	100	—	11	—	—	—	—	—	—	—	—	—
302–151	9	—	—	—	—	—	—	—	3	—	—	—	1
303—50	13	—	—	—	—	—	—	—	—	—	—	—	
Total		100	100	88	(11)	100	(7)	30	8	100	100	20	90

frequency of chromosome D.

Ty	Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
7	—	20	9	22	48	100	26	34	10	14	20	18	35	8
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	22	—	2
7	2	1	1	—	—	—	—	—	—	—	—	—	—	-
156	34	76	90	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	-	
2	—	1	—	—	—	—	—	—	—	—	—	—	-	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	
		—	—	—	—	—	—	—	—	—	—	—	2	-
—	—	1	—	—	—	—	—	—	—	—	—	—	—	
—	—	1	—	—	—	—	—	—	—	—	—	—	—	-
-	—	—	—	—	—	—	—	—	—	—	—	—	—	
	—	—	—	—	—	—	—	—	—	—	—	—	—	-
-	—	—	—	—	—	—	—	—	—	—	—			
	—	—	—	—	—	—	—	—	—	—	—	—	11	9
-	—	—	—	—	—	—	—	—	—	—	—	—	2	1
172	36	100	100	22	48	100	26	34	10	14	20	40	50	20

frequency of chromosome E.

Srr N	Hr	Ty	Ms	Ak	Ot	Fd	Kk	Rf	Od	Kb	Sn	Ke	On	Np	Sg	My
—	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	14
1	—	8	—	1	—	—	—	—	—	—	—	—	—	—	—	-
8	3	76	12	15	30	—	—	—	—	—	—	—	—	14	7	
—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	-
36	45	7	20	40	36	—	—	—	—	—	—	—	—	11	3	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
1	1	1	—	1	—	—	—	—	—	—	—	—	—	—	—	-
1	—	5	—	2	—	—	—	—	—	—	—	—	—	—	—	—
26	10	—	3	22	—	—	—	—	—	—	—	—	—	—	—	-
	-	-	—	—	—	—	—	—	—	—	—	—	—	—	—	—
			—	1	—	—	—	—	—	—	—	—	—	—	—	-
				—	—	—	—	—	2	6	1	—	—	—	—	—
17	6	67	1	5	34	—	—	—	—	8	9	14	20	15	40	6
—	1	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—
		-	—	1	—	—	—	—	—	—	—	—	—	—	—	-
		-	—	—	—	22	48	100	24	20	—	—	—	—	—	-
—	—	2	—	1	—	—	—	—	—	—	—					
90	66	172	36	100	100	22	48	100	26	34	10	14	20	40	50	20

Genetic Analysis of Varietal Differentiation in Cereals. VII. Interrelationships between Potential Variabilities and Environmental Conditions.*

By

Kanji GOTOH

(Received June 28, 1956)

A series of experiments have been conducted by the author in order to analyze the varietal differentiation in cereals from the viewpoint of population genetics. In a few examined varieties whose original stocks must have been heterogeneous or heterozygous, differences in certain characters were found between several geographical strains.

It is well known that natural selection plays a significant role in the speciation of wild plants. However, only a few experiments have thrown light upon the direct role of natural selection in the varietal differentiation of of cultivated plants.

The experiments of this series have been designed and carried out in order to confirm the effects of natural selection upon varietal differentiation in a barley variety, "Hosogara No. 2". Moreover, some general and important problems connected with varietal differentiation are discussed from the standpoint of practical plant breeding.

1. Comparative experiments with local strains concerning cold resistance

Local strains of "Hosogara No. 2" were classified into 3 groups, X, Y and Z, basing on their morphological similarities and growth habit (Gotoh (3) and (4)). X group which has evolved in Hokkaido, included *A*, *A*–*S* and *C* strains. *A* strain is a representative of this group. *A*–*S* and *C* strains were artificially separated from *A*, the former as spring and the latter as winter type. *D* strain obtained from Aomori Prefectural Agr. Exp. Sta. belonged to Y group and was of medium winter type. All population samples (e.g. *E*, *F* and *G* strains) obtained from south-western locations showed the features of so-called Z type, and were homogeneous regarding spring habit.

The spring habit strain of the X group (*A*–*S*) and those of the Z group (*E*, *F* and *G*) were different upon visual inspection and in competitive ability (Gotoh (4) and (5)).

* Contributions from the National Institute of Genetics, Misima, Japan. No. 163.
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Gotoh (4) found that the population sample of *A* strain included three groups of genotypes, X, Y and Z, in a proportion of 91.0 %, 7.2 % and 1.8 %, respectively. These figures show that only a few Z type plants were included in X group. It was suspected that the Z plants had been eliminated to a great extent from X group under the severe winter conditions of Hokkaido.

In order to compare the local strains for their cold resistance and to attack the relations between cold resistance and geographic distribution of Z group, a preliminary experiment with three strains, *C*, *D* and *E*, was carried out in 1953 at the National Institute of Genetics, Misima. Seedlings 12 days after germination were exposed during 10 hrs to low temperature (−7.5°C) in a refrigerator. After 2 weeks the surviving plants were counted. The survival rate of *C*, *D* and *E* strains was 81.1 %, 80.6 % and 70.2 %, respectively.

Furthermore, two designs of experiments were conducted at the Kitami Branch of Hokkaido Agr. Exp. Sta., Kitami, Hokkaido, during two seasons, 1953–54 and 1954–55. 6 strains, *C*, *A–S*, *D*, *E*, *F*, and *G*, were examined: the sowing date was September 20th in both seasons. Under the first design, plants were grown in a randomized block arrangement with 4 replications according to the usual cultivation methods. The plants were counted before and after overwintering, and the proportion of overwintered plants to the total plant number counted before winter was calculated.

Table 1. Comparison of rates of overwintering plants.

Name of strains	1953–54		1954–55	
	a*	b**	a	b
C	154	85.1	736	91.3
A–S	174	79.7	757	90.4
D	181	94.5	748	94.7
E	173	74.3	725	86.6
F	162	74.3	717	88.9
G	173	79.3	753	91.3

* a shows number of plants before overwinter.
** b shows rate of overwintering plants in per cent.

As seen from Table 1, injury inflicted during 2 winters was not striking, and the difference between the strains in survival rate was insignificant. According to the results of analysis of variance the difference between the two years was highly significant, while differences between strains and interaction between strains and years were non-significant. However, the difference between X and Y group (*C*, *A–S*, *D*) and Z group (*E*, *F*, *G*) was significant at the 5 % level. Since the table order of the strains with respect to survival rate is similar in both years, it seems that there was a difference between the strains in this character, although it was non-significant.

In order to test the resistance of the strains against extremely low temperatures, an experiment was conducted in 1954. Plants were grown in a

frame in a randomized block arrangement with 3 replications. A roof was placed over the frame before the plants were covered with snow. In the night of Jan. 26th, the roof with accumulated snow was removed, and the plants were exposed for about an hour to −23.6°C. Thereupon, the roof was restored. In spring (Apr. 6th), the degree of injury was determined by grading the plants from 0 (no injury) to 6 (completely destroyed). The degree of injury in each strain was calculated by the following formula.

$$\frac{\text{Total sum of the degrees given within a strain}}{6 \times \text{total number of plants}} \times 100$$

The degree of injury averaged for three blocks for the strains, *C*, *A–S*, *D*, *E*, *F* and *G* was 89.0, 96.5, 98.8, 99.0, 97.7 and 95.6 %, respectively. The injury in the *C* strain was the smallest and that suffered by all others was almost the same. It may be assumed, then, that cold resistance of *C* strain was superior to that of the other strains under temperatures as low as −23.6°C.

Since the former experiment was conducted in too mild winters and the latter was done under too severe conditions, the difference between the strains was not striking. However, the results of both experiments indicate that cold resistance of Z type plants is lower than that of the winter habit plants of X group, which might have resulted in the almost complete elimination of Z type plants from the population of X group during the long period of multiplication.

2. Potential variability in the response to the critical sowing date

The degrees of winter habit of varieties in cereals are usually determined by spring sowing experiments, in which seeds of a given variety are sown periodically from early spring until summer. When seeding is carried out at a later date than the specific so-called critical sowing date, winter habit plants cannot produce ears. According to Yamamoto and Obara (30), when the date of sowing approached the critical period, in a few barley varieties plants both capable and not capable to produce ears were found, and their date of heading was irregular. As pointed out in a previous report (Gotoh (4)), such a phenomenon was observed in *D* strain of "Hosogara No. 2" in a spring sowing experiment, in which the difference between the lines in the number of heading plants was striking.

An experiment was carried out to find out whether the differences between the lines were governed by genetic or environmental factors. 100 lines of *D* strain obtained from threshing on individual plant level in 1952, were examined repeatedly in 1954 and 1955. 10 plants per line were grown 10 cm apart in rows spaced 30 cm with no replication in 1954 and in a randomized block arrangement with three replications in 1955. Sowing date was Feb. 27th in 1954 and Feb. 28th in 1955. In both experiments the number of heading plants was counted for each line in June 30th.

Upon visual inspection, *D* population was homogeneous in several agronomic characters, such as type of seedlings and young plants and in the date of heading, when grown under normal conditions. However, polygenic variability was found among the lines in ear length and ear density (Gotoh (3)).

According to Gotoh (4), *D* population consisted of 99.4 % of medium winter habit and 0.6 % of spring habit plants. It may be worth adding here some date regarding the degree of winter habit of *D* population. Before sowing, seeds of winter habit plants were kept 20 and 45 days at 0°C. Germination and further development took place in the air-conditioned greenhouse at 20°C under long day treatment. On the 85th day after the start of long day treatment, none of the plants obtained from seeds kept 20 days at 0°C produced ears, while all obtained from seeds treated 45 days produced ears on the 40th day after the start of long day treatment; at that time the number of leaves on the stem was 6. Thus, it was found that the winter habit of *D* population can be changed to spring habit by exposing the seeds to a low temperature (0°C) for 45 days.

Table 2 shows the number of heading plants per line and the number of lines in each class in 1954 and 1955. As seen from the table, a wide range of variation in the number of heading plants was found among the lines. The result of variance analysis for the date in 1955 is presented in Table 3.

Table 2. Variation in number of heading plants among lines.

1954		1955	
Num. of heading plants	Number of lines	Num. of heading plants	Number of lines
0	3	0	8
1	8	1～ 3	31
2	13	4～ 6	21
3	17	7～ 9	18
4	21	10～12	6
5	9	13～15	9
6	8	16～18	5
7	9	19～21	1
8	5	22～24	0
9	5	25～27	0
10	2	28～30	1
Tot.	100	Tot.	100

The difference between the lines was sigfinicant at the 1 % level, while variance due to replications was non-significant. It was concluded that the difference between the lines in the number of heading plants was largely governed by genetic factors. Basing on the data of two years, the correlation coefficient between both years was calculated to be +0.2584. Though the correlation coefficient was highly significant, it was very low. It may be surmized that interaction between years obscured the interrelationships between the lines in their order concerning the number of heading plants. However, so far as production of ears in most or none of plants of a line is concerned, the data

Table 3. Analysis of variance (1955)

Sourse of variation	d. f.	m. s.
Between lines	99	9.70
Between blocks	2	5.63
Error	198	1.90

of both years corresponded fairly well. In the experiments mentioned above, the main factors controlling ear emergency may be low temperature and short day in early spring, but it cannot be overlooked that response to nutrition and drought could be different between the lines.

The variability found in *D* population in the response of lines to the critical sowing date will be discussed in a later section.

3. Effect of sowing date on the frequency of spring habit plants

Without exposure to low temperature or short winter day, the winter habit plants are not capable to attain their full growth and to produce ears, while the spring habit plants do not need either the former or the latter factor for ear emergency. Consequently, in the southern locations, when the winter is warm or the sowing date is delayed, the spring habit type may be more favored than the winter type in respect to seed multiplication.

An experiment was planned to inquire into the actual increase of seed production of the spring habit type accompanying a delayed sowing date. Four artificial mixtures and a hybrid population of local strains of "Hosogara No. 2" were examined at the Nat. Inst. of Genet., Misima, in 1954. In the first experiment the following four mixtures were used. Seeds of *A*–*S*, *E*, *F* and *G* strains were mixed with those of *C* strain in equal numbers. The former strains are of spring habit type and the latter is an extreme winter type. Date of sowing was Nov. 26th and Dec. 26th in 1954 and Jan. 26th in 1955. The plots consisted of two rows 4 m long, spaced 50 cm apart. After the harvest and threshing at the plot level, randomly selected 150 seeds per plot were sown in a greenhouse maintained at over 15°C. From the beginning of the experiment, the plants were illuminated by electric lamps through night. On the 35th day after sowing, the heading plants were counted in each plot.

Table 4. Frequency of spring habit plants in per cent.

Date of sowing \ Mixtures	*C*+*A*–*S*	*C*+*E*	*C*+*F*	*C*+*G*
Nov. 26, 1954	51	46	62	58
Dec. 26, 1954	56	58	68	71
Jan. 26, 1955	86	69	75	82

According to Table 4, differences in frequencies of spring and winter habit types were not striking in the plots sown at a normal sowing date (Nov. 26th), whereas in the plots sown later, spring habit plants increased in each mixture. The predominance of spring habit plants (69~89 % of the populations) in plots sown at the latest date is very striking. In the same manner, the second experiment was conducted with an F_2 hybrid population from the cross $E \times C$. In the air-conditioned greenhouse maintained at 20°C

under long-day condition the F_1 hybrid of this cross showed winter habit, like *C* strain, and the ratio of 3 (winter) to 1 (spring) was obtained for the F_2 population. After the harvest, F_3 plants were tested under long day condition.

As seen from the table, the frequency of spring habit plants is almost identical with the theoretical expectation (37.5 %) in the plots of Nov. 26th and Dec. 26th, while in the latest plot it attains 46 %. The data presented herein show that the frequencies of spring habit plants in the F_3 generation were affected by delayed sowing. This indicates that spring habit plants should be endowed with a selective advantage in the delayed sowing against the winter habit ones. Thus, it seems likely that such an advantage would have, more or lsss, contribute in the process of differentiation of Z group (whicn consists of spring habit plants) to the southern locations.

Table 5. Frequency of spring habit plants in a F_3 hybrid population.

Date of sowing	Freq. (%)
Nov. 26	36
Dec. 26	36
Jan. 26	46

4. Causal factors concerning varietal differentiation

It is well known that in certain varieties even of selfpollinating cultivated plants, varietal differentiation sometimes occurs during the period of multiplication. What is the fundamental source of varietal differentiation in such plants?

First, varieties which seem to have been heterogeneous at the start sometimes include a wealth of genetic variability, and local strains differing in their genetic composition are found in such varieties, like in the cases of "Hosogara No. 2" and "Iwate Mensury No. 2" (Gotoh (3), (4), (5) and (8)). In some oat varieties a similar variability was found by Granhall (9) and Wallace et al. (28).

Secondly, in varieties, produced from a cross, which are originally heterozygous, segregation will inevitably take place after release. Such a case was found in a beer-barley, "Hakata No. 2" (Gotoh (6)). In this variety the difference between local strains in ear length was striking. Since fixation of segregants for polygenic or invisible characters is far more difficult than it has been assumed, residual heterozygosity in certain characters may frequently be the cause of varietal differentiation.

In connection with the mutability of domesticated species, Mangelsdorf (20) stated that "Pure lines of self-fertilized plants such as wheat, oats and barley do not long remain pure. Although appearing to retain their uniformity in their principal morphological characteristics, they often prove to be heterozygous in resistance and susceptibility to new races of rust and other diseases when experimentally inoculated." A similar phenomenon was foun in *D* strain of "Hosogara No. 2" in the spring sowing experiment, as shown in section 2. It is well known that such a situation has been found in inbred lines of mice or chickens.

Thirdly, the propagation of mutants occurs during the period of seed multiplication. Moreover, the so-called pure lines of self-pollinating plants or inbred lines of cross-pollinating plants seem to increase their genetic variability through the occurrence of small mutations, as suggested by Jones (19). It is highly probable that polygenic mutations would occur more frequently than those of major genes, and they would play a significant role in varietal differentiation. However, regarding the frequency of polygenic mutations and preservation of polygenic mutants in populations of cultivated plants, we have few data at present, because of difficulties in detecting such mutants.

On the other hand, in polyploid plants, such as wheat or oats, the occurrence of off-types must be considered. Tall and short off-type plants found in a wheat variety, "Saitama No. 27" (Gotoh (7)), may belong to this category. Tall off-types in Clinton oats reported by Morey (21) are a well known case.

Finally, the effects of natural intra- and inter-varietal crosses and contamination by seeds of other varieties must be taken into consideration. According to Harrington (14) and others, the proportion of natural crosses is low-less than 0.5 % in most varieties of cereals. However, Robertson and Coleman (24) found in one barley variety as much as 21 % of natural crosses in one year, and according to them, there seems to be a greater difference in this respect between the varieties than between the seasons. Therefore, in mixed varieties, like "Hosogara No. 2" or "Iwate Mensury No. 2", if the frequency of natural crosses were high, then they might play a significant role in increasing intra-varietal variability.

As a matter of course, genetic drift would greatly influence differentiation of varieties in cultivated plants. This problem will be discussed later (Section 6).

Let us consider the causes of the phenomenon presented in section 2. *D* population of barley variety, "Hosogara No. 2", included a fairly wide range of variability in response to the sowing date in spring.

In first place, we can assume that variability may be due to heterozygosity which remained in the population: in other words, variability found between and within lines may be due to polygenic segregation. But from the theoretical point of view, it is difficult to explain residual heterozygosity in this variety, since it has been cultivated during more than 30 years after its release. However, this population has been never subjected to artificial selection under such environmental conditions as those of the experiment. Thus, it seems plausible that variability found in our experiments is due to polygenic segregations.

Secondly, we can assume that variability may be due to heterogeneity in response to abnormal conditions in spring. In other words, some of the lines would have a genotype allowing them to produce ears in most of the plants under such conditions, while the genotype of others would enable them to produce ears only in a few plants or none. In this case, variation found between the lines would be governed by polygenes.

Which assumtion is correct? What kind of mechanism is concerned with

the maintenance of heterozygosity or heterogeneity in a population during such a long period of multiplication?

These problems are of great interest from the viewpoint of population genetics. Furthermore, there is no doubt that natural selection could affect powerfully such populations, as the *D* strain of "Hosogara No. 2",, which are heterozygous or heterogenecus in respect to certain polygenic characters.

5. Active agents inducing varietal differentiation

In this connection the question arises in what manner does natural selection affect populations of cultivated plants. The significant role of growth habit in varietal differentiation has been pointed out by Gotoh ((4) and (8)).

According to Akihama (2), wheat varieties having long postharvest dormancy are cultivated in the south-western localities of Japan, where there is much rain at the time of harvest and wheat sometimes incurs the risk of germination before threshing, whereas the farmer in the northern parts is usually not threatened by such a phenomenon.

If certain varieties which include plants with weak dormancy were brought into the south-western districts, varietal differentiation due to elimination of such plants would inevitably take place. On the other hand, in the northern districts, as Hokkaido, where seeds of winter barley and wheat are sown about 40 days after the harvest, plants having extremely strong dormancy may be eliminated before stand.

O'Kelly (23) examined during 10 years changes occurring within a variety of cotton, and he found a progressive increase in the proportion of naked seeds in the later generations, and also, a progressive decrease in lint percentage and ball weight, presumably accompanying the increase of naked seeds. In fact, the naked seeds showed good germination even under dry condition and the seedlings attained 1~2 weeks growth, before fuzzy seeds started to germinate after a rainfall. It seems to be a suitable example which shows that drought resistance is closely connected whith varietal differentiation. In this case natural selection acts powerfully soon after sowing.

After the plants stand, the variability of population in cold resistance, in resistance to certain diseases or to races of a disease, and in resistance to insects may be regarded as the source, on which natural selection acts.

According to Sylvén (27), when two strains of white clover of Danish and German origin which were hererozygous in their genetic composition were planted in the more severe climate of southern Sweden, they became adjusted to the climate over a period of two years through selective elimination of the less hardy plants. A similar situation may occur, also, in self-pollinating plants.

During the period of growth, competition between plants growing side by side may take place. Competition in cultivated plants is generally restricted to intra-varietal level. In this context the so-called survival values should be considered, which may be treated mathematically as a function of competitive

and propagating abilities. The relation between these two factors were briefly mentioned by Gotoh (5). Moreover, both factors are at the same time a function of environmental factors.

As reported by Gustafsson and Nybom (12), in competitive planting of a hybrid population including besides a mutant strain, *bright green 4* (induced by X-ray from Bonus barley), the original strain and the hybrid, the mutant type tended to produce more numerous and heavier kernels in dense stands than the original type, and the reverse was true of its behavior in thin stands.

Generally speaking, this indicates that the competitive and propagating abilities of certain varieties, segregants or mutants may be modified by the density of planting. Consequently, the dynamic nature of survival value may be exactly explored by examining the materials in single and mixed stands under a variety of contolled environmental conditions.

If a population includes genetic variability regarding competitive and propagating abilities, its constitution in the following generations may change through the decrease of less adaptable plants, poorer propagators and weaker competitors.

Temperature response of plants during the period of growth seems to be one of the most powerful factors governing varietal differentiation, like growth habit and photoperiodic response, though in this respect only few informations have been so far obtained.

According to Went (29), "Fruit set and seed production in tomatoes is dependent upon a very narrow range of night temperature," and ".....There are rather slight differences in optimal temperature for fruit set between varieties." When two varieties of tomatoes were planted in two locations with a difference in night temperature of only about 3°C, their fruit yields were reversed in each location. Such a situation may take place with respect to seed production among plants constituting a population in other cultivated plants. Thus, differences in temperature response of the population components will exert a controlling effect upon varietal differentiation.

Finally, we cannot overlook the effect of soil conditions and manures upon varietal differentiation. As is well known, tolerance of wheat varieties to acid soils varies to a considerable extent, and Neenan (22) found among four wheat varieties a differential response to two factors of the soil acidity complex, namely aluminum and manganese toxicity.

It has been reported by Gregory and Crowther ((10) and (11)) that there are differences between varieties in their ability to utilize the applied fertilizers. Artificial selection concerning the soil acidity complex or the ability to utilize the fertilizers seems to be very difficult in practice. Consequently, genetic variability may remain in varieties which have never been subjected to artificial selection for these characters. Thus, varietal differentiation due to soils (acid or alkaline, poor or rich, etc.) and due to fertilizers (in quantitative and qualitative sense) may occur in varieties preserving a considerable degree of heterogeneity or heterozygosity regarding the response to soils and fertilizers.

6. Varietal differentiation and plant breeding

Degeneration of varieties has been for many years a widely discussed problem. But only insufficient explanation of this phenomenon has been obtained. This problem has been dealt with in my experiments and I believe that light can be shed upon some points of this phenomenon with the help of theoretical considerations derived from experiments conducted from the viewpoint of population genetics.

As a matter of course, cultivated plants are exposed to many kinds of limitations by man. Especially, varieties have been occasionally maintained as uniformly small populations without any consideration of their different genetic variability. The smaller is the population size, the greater is the danger of elimination of rare genotypes and the risk of increasing certain mutants or recombinants due to natural crosses with other varieties. Furthermore, sowing date and harvesting date may affect unintentionally varietal differentiation. For example, frequencies of spring habit plants become higher with the delay of the sowing date, as shown in section 3. In certain populations including variability in earliness, extremely early or late genotypes may be eliminated by a limiting harvest date.

Artificial control of planting density could also affect population structure of varieties, through an increase of plants which prefer a given density, as briefly mentioned in the former section in connection with a variation in survival value due to density of planting.

Moreover, it may be expected that unconscious artificial selection in the case of seed preference by man, and mechanical selection under threshing or screening by machine will, also, occur.

Thus, varietal differentiation may be unconsciously accelerated or exaggerated under the control of man.

The nature and process of varietal differentiation may be analyzed by examination of the shift of artificial mixtures of varieties or hybrid populations exposed to various environmental conditions. Such procedure was employed in my experiments as demonstrated in section 3.

In this field of research, Harlan and Martini (13) and Suneson (26) have conducted extensive research using barley varieties.

The essential part of the data of their experiments may be generalized as follows.

1) In an artificial mixture of varieties (in certain environments) some predominate during the period of multiplication and others are thoroughly eliminated or occupy only a small part of the given mixture.

2) Some varieties constituting certain mixtures are capable to become adapted to a wide range of locations, where others are adaptable to restricted locations.

3) There are environments, in which a number of varieties apt to be preserved, and the reverse is true of other environments.

These findings throw light upon the process of micro-evolution, and they

clearly show how rapidly natural selection can affect the population structure of cultivated plants.

It seems to be of interest to compare in detail the characteristics of varieties and environments basing on experiences like those mentioned above. Through similar experiments designed under the above assumptions, the causal factors and actual agents of varietal differentiation may be explored, and fundamental valuable information regarding practical breeding may be deduced.

In order to investigate the effect of natural selection upon a bulk hybrid rice population, Adair and Jones (1) examined the characteristics of surviving plants in populations grown each under three different environmental conditions during a period of several years. The effect of environment was striking upon the number of days from seeding to heading, plant height, seed shape and so on. It may be worth securing in such a manner genic systems affected directly by natural selection in various environments. Such information would serve as a reference for plant breeders.

Special attention must be paid to the characteristics of varieties dealt with in practice. They are sometimes mixtures and include a wealth of genotypes as reported by Gotoh ((4), (5), (6) and (8)). In such cases it is necessary to maintain them as considerably large population, in order to prevent the shift of their constitution due to genetic drift.

At the same time, plant breeders must take care of the variability of varieties, employed as parents in crossing. Moreover, when one intends to use a variety of wheat or oats for crossing, he should examine whether it produces off-types or rogues and speltoid mutants. As has been pointed out in section 4, occurrence of off-type plants in wheat or oats may become one of the cause of varietal differentiation. It may be assumed that the behavior of varieties producing often off-type plants is of genetic nature. When varieties with such behavior were used as parents in crossing, similar segregants might occur in later generations of the hybrid.

The above considerations show how important it is to be acquainted with the mechanism and causes of varietal differentiation of cultivated plants. It might prevent unwelcome differentiation and degeneration.

7. Problem of multiline-variety

When a variety is cultivated in a wide range of areas, its plasticity should be examined under various ecological conditions. In this respect, Hunter (17) pointed out that "if the variety under propagation is intended for wide geographical distribution it is always worth considering whether a mixture of very like forms may not meet the necessities of the position better than a single line. It is possible that a mixture of forms of like morphology but of differing physiological constitution might possess a much wider range of adaptability and, hence, on the average, respond to the varied conditions under which it will be grown better than a single line." In this connection, Stebbins (25) stated that "a wide range of tolerance, and therefore a wide geographic distribution, may be acquired by a species both through the possession of a

wide variety of different genotypes and through existence of certain individual genotypes which by themselves have particularly wide range of tolerance."

Hiesey (16) made a similar statement for a wild population under the pressure of natural selection.

The above mentioned idea regarding diversification of varieties, especially in self-fertilizing plant, is an old one. Heinish (15) conducted a classical experiment in order to compare the yield of pure lines and their mixtures. The performance of the latter were good in unfavorable seasons.

Recently, Jensen (18) discussed the principles and methods of diversification at the intra-varietal level. Data presented by him showed that, in general, artificial mixtures of different species or varieties yielded more than their single stands, though the differences were statistically non-significant. He discussed in detail the characteristics and the advantage of a multiline-variety with special reference to yield stability under various kinds of diseases and their physiological races.

The most difficult problem in breeding a multiline-variety is the proportion and nature of component lines, and the population shift during the period of multiplication. It may be desirable that a multiline-variety be synthesized from lines with a similar degree of competitive and propagating abilities under various environmental conditions, and that it be homogeneous in phenotypic features, such as plant height, date of heading, date of maturity, etc., on the other hand, that it be heterogeneous in respect to response to various kinds of environmental factors, and in respect to resistance for diseases and so forth.

As has been pointed out by Gotoh (4), the proportion of spring and winter habit plants of the local strains of the variety, "Hosogara No. 2", which has evolved in Hokkaido, remained almost the same during over 15 years of multiplication. It may be assumed that the above mentioned strain has attained its equibrium whose mechanism should be explored in the future. Suitable experiments would yield fundamental informations for the breeding of multiline-varieties.

Summary

In order to find out the direct effects of natural selection upon the differentiation of the barley variety, "Hosogara No. 2", a series of experiments with its local strains have been carried out in the air-conditioned greenhouse of the National Institute of Genetics, Misima and at the Kitami Branch of Hokkaido Agr. Exp. Sta., Kitami, Hokkaido. Furthermore, it was attempted to throw light upon some aspects of the mechanism of varietal differentiation in cereals.

The experimental results and the essential points of the considerations may be summarized as follows:

1. Survival rate of overwintering plants in the southern local strains (Z group) was lower than that of the winter habit plants of X group (which has evolved in Hokkaido), which might have resulted in an almost complete

elimination of Z type plants from the populations of X group under the severe winter conditions of Hokkaido.

2. *D* strain of "Hosogara No. 2" which has been cultivated during more than 30 years since its release, was homogeneous in several agronomic characters upon visual inspection. But when it was sown at the end of February, variability regarding the number of heading plants was found between the lines. It was concluded that this variability was of genetic nature. Causes of the phenomenon were discussed in section 4.

3. Four artificial mixtures of winter type plants with spring type plants, and an F_2 hybrid population obtained from a cross between both were sown at three dates namely at the end of November, December and January.

The predominance of spring type plants in the plots sown at the latest date was very striking. It was concluded that Z group which consists of spring type plants could have been evolved in the southern locations owing to their advantage over the winter type, resulting in the elimination of the latter.

4. Residual heterogeneity and heterozygosity of the original stocks are considered to be responsible for varietal differentiation. It was considered that polygenic mutations, occurrence of off-type plants in polyploid plants and natural crossing might play a significant role in increasing intra-varietal variability.

5. It was discussed in what manner varieties of cultivated plants differentiate, Genetic variability in respect to the following characters was regarded as the responsible active agent: growth habit, photoperiodic response, temperature response, dormancy, drought and cold resistance, disease resistance, and response to soils and fertilizers.

6. Varietal differentiation of cultivated plants could be accelerated by man. Special attention was focussed upon the limitation by population size used in maintaining the materials, upon artificial control of planting density, and upon unconscious artificial selection or unintentional mechanical screening at the time of threshing and other preparatory procedures. In this connection, it was called to mind that natural selection will affect powerfully artificial mixtures or hybrid populations.

7. Several problems concerning diversification of varieties in self-pollinating plants were presented and discussed. It was concluded that the information regarding the mechanism and the causes of varietal differentiation may offer valuable suggestions for methods of synthesizing and handling multiline-varieties.

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Cytogenetical Studies on the Intergeneric F_1 Hybrids Raised between Emmer Wheat and Two Species of *Secale*.

By

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I. Introduction

Among the cytogenetical studies on the wheat-rye intergeneric F_1 hybrids, those carried out on the hybrids between Emmer wheat and *Secale* are few, compared with those hybrids between Dinkel wheat and *Secale* species, and especially few concerning the hybrids between Emmer wheat and *Secale africanum* and *montanum*, as we see so far from the work by Longley and Sando (1930).

As one of a series of cytogenetical studies on intergeneric hybrids between *Triticum* and *Secale*, the present author tried to produce some hybrids from Emmer wheat and two species of *Secale* (*africanum* and *montanum*) in 1952～1954. Some cytogenetical studies on the F_1 plants were made and the results obtained will be dealt with in this paper.

II. Materials and methods

Five species of Emmer wheat were used as mother plants in this hybridization. They were *Triticum durum, turgidum, dicoccum* var. *atratum, pyramidale* and *polonicum*. The seed of *Secale africanum* and *S. montanum* employed as the pollen parent were kindly sent from Prof. Arne Müntzing of Lund University, Sweden.

The root tip cells and anthers fixed with either Nawashin's or Carnoy's fluid were used for the cytological study of somatic chromosomes and meiosis in the same method as used in the previous papers by the present author on wheat-rye hybrids. Original magnifiction of figures is ×2300 for the somatic chromosomes and ×2300 for the meiotic chromosomes.

III. Results of hybridization

The intergeneric hybridization between Emmer wheat and each of the two species of *Secale africanum* and *montanum*, was carried out in 1952～1954, and the results of the hybridization were as shown in Table 1.

Table 1. The results of hybridization between Emmer wheat and each of 2 species of *Secale*.

Combination of hybridization	Number of spikes	Number of spikelets	Number of kernels	Percentage of seed setting
T. durum × *S. africanum*	59	1301	182	13.982
T. turgidum × "	180	3729	209	5.605
**T. polonicum* × "	67	1206	11	0.912
**T. pyramidale* × "	60	1036	35	3.378
T. dicoccum × "	99	2135	19	0.890
T. durum × *S. montanum*	58	1261	362	28.707
T. turgidum × "	138	2859	375	13.116
T. polonicum × "	89	1551	88	5.665
T. pyramidale × "	58	815	157	19.264
T. dicoccum × "	44	775	61	7.858

* The results were reported by the present author previously.

As shown in Table 1, the seed fertility of the two combinations of the hybrids between *T. durum* and two species of *Secale* (*africanum* and *montanum*) showed the highest among the hybrids involving Emmer wheat, and next came those of the hybrids with *turgidum* and *pyramidale*, and finally those of the hybrids with *polonicum* and with *dicoccum* were lowest.

The diversity of percentage seems to depend on the nature of the species of wheat employed. According to the present author (1956), the percentage of seed setting in the F_1 plants of *T. persicum* with *S. africanum* and *montanum* was 20.539 and 30.739 respectively, and these values were superior to those of the present results. Thus the percentage of seed fertility of the intergeneric hybrids differs exceedingly among different species of wheat.

Table 2. Germination of the F_1 seeds.

Combination of hybridization	Number of seeds sown	Number of germinated seeds	Percentage of germination	Number of plants died in winter	Number of matured F_1 plants	Percentage of F_1 plants to pollinated flowers
T. durum × *S. africanum*	100	44	44.00	8	36	5.036
T. turgidum × "	100	39	39.00	6	33	1.850
T. polonicum × "	11	8	72.73	4	4	0.332
T. pyramidale × "	35	5	14.27	3	2	0.193
T. dicoccum × "	19	15	83,75	4	11	0,515
T. durum × *S. montanum*	100	41	41.00	5	36	10,335
T. turgidum × "	100	60	60.00	34	26	3.410
T. polonicum × "	88	48	54,55	22	26	1.670
T. pyramidale × "	157	5	3.12	2	3	0.368
T. dicoccum × "	61	7	11.48	3	4	0,516

The seeds obtained by this hybridization were sown in October of the same year and some F_1 plants were obtained. The percentage of F_1 plants to the number of seeds sown is shown in Table 2.

As seen in Table 2, the ratio of the F_1 plants to the number of pollinated flowers were almost parallel to the results of seed setting, while the germination of the F_1 seed raised by the hybridization with *T. pyramidale* showed very low percentage; therefore the percentage of F_1 to the pollinated flowers in this hybrid was rather lower as compared with the seed setting percentage. It seems to indicate from the results mentioned above, that the affinity between the species is more powerful in the combination between Emmer wheat and *S. montanum* than that between Emmer wheat and *S. africanum*.

For convenience, the eight hybrids raised by hybridization between the plants mentioned above, will, hereafter, be represented by the following symbols: *T. durum*×*S. africanum*=TdurSaF_1, *T. turgidum*×*S. africanum*=Ttur SaF_1, *T. dicoccum* var. *atratum*×*S. africanum*=TdicSaF_1, *T. durum*×*S. montanum* =TdurSmF_1, *T. turgidum*×*S. montanum*=TturSmF_1, *T. polonicum*×*S. montanum*=TpolSmF_1, *T. pyramidale*×*S. montanum*=TpySmF_1, and *T. dicoccum* var. *atratum*×*S. montanum*=TdicSmF_1.

IV. External characters of F_1 plants

As can be seen in Table 2, the number of mature F_1 plants, varied from 2 in the case of *T. pyramidale*×*S. africanum*, to 36 in the case of *T. durum*×*S.*

Table 3. External characters of the F_1 hybrids and their parental plants.

Characters / Plants	Length of culms cm	Length of spikes cm	Length of awns cm	Number of spikelets per spike	Spike density	Number of flowers per spikelet	Number of tillerings
T. durum	143 58	6.54	8.87	21.23	3.25	4	
T. turgidum	156.92	6.78	7.76	25.16	3.71	4	
T. polonicum	142,38	14.70	12.55	21.45	1.46	3	30.70
T. pyramidale	125.40	5.58	14.96	20.64	3.69	4	39.83
T. dicoccum	131,46	10.37	10.78	29.05	2.80	3	45.44
S. africanum	130.40	13.60	0.00	50.40	3.71	2	
S. montanum	111,00	15,23	1.23	44.60	2.93	2	
TdurSaF_1	165.02	13.00	4.94	36.36	2.71	4	50.60
TturSaF_1	160.60	15.11	6.18	41.18	2.73	4	76.40
TdicSaF_1	152.00	14.11	4.11	40.86	2.90	4	83.00
TdurSmF_1	159,30	13.58	5.78	37.20	2.74	4	79.20
TturSmF_1	167.70	14.74	6.13	42.68	2.90	4	71.50
TpolSmF_1	149.41	21.68	6.29	37.02	1.71	4	65.42
TpySmF_1	118.30	15.17	7.95	36.33	2.39	3	83.00
TdicSmF_1	158,38	16.15	5.08	40.43	2.51	4	82.75

africanum and *T. durum*×*S. montanum*. Some individual differences were observed in the external characteristics of the F_1 plants, even of the same combination, though not so remarkable. The external characteristics of these F_1 plants and their parent plants are shown in Table 3 and photos 1~6.

Photo. 1~3. Spikes of F_1 and its parents, from left to right: mother plant, F_1 and pollen plant. $\times$ca. $\frac{1}{2}\times\frac{3}{4}$.

1. *T. durum*, TdurSaF_1 and *S. africanum*.
2. *T. turgidum*, TturSaF_1 and *S. africanum*.
3. *T. dicoccum* var. *atratum*, TdicSaF_1 and *S. africanum*.

As shown in Table 3 and photos 1~6, the culm height of all the F_1 plants were superior to that of both parents, except TpySmF_1 whose value lies between those of the parents. In some cases the lengths of spikes were superior to those of both parents (TpolSmF_1, TdicSmF_1), and in the other ones more closely resembled the pollen parents. The lengths of awn of F_1 were almost intermediate between the parents in every combination. The number of spikelets per spike of F_1 plants somewhat closely resembled that of the pollen parents rather than intermediate between the parents. But the spikelet density of F_1

Photo. 4~6. Spikes of F_1 and its parents, from left to right: mother plant, F_1 and pollen plant.

4. *T. polonicum*, TpolSmF_1 and *S. montanum*. $\times$ca. $\times \frac{2}{5} \times \frac{3}{4}$.

5. *T. pyramidale*, TpySmF_1 and *S. montanum*. $\times$ca. $\frac{3}{5} \times \frac{3}{4}$.

6. *T. dicoccum* var. *atratum*, TdicSmF_1 and *S. montanum*. $\times$ca. $\frac{3}{5} \times \frac{3}{4}$.

is inferior to that of both parents in every combination. The number of flowers per spikelet of the F_1 is quite the same as that of the mother plants. When ripening the spikelets of all F_1 plants become brittle. Similar cases were previousely reported by the present author in the F_1 obtained from wheat crossed with *S. africanum* and with *S. montanum*. Generally speaking, although the F_1 plants possess external characteristics of both parents, they resemble somewhat more closely the pollen parents.

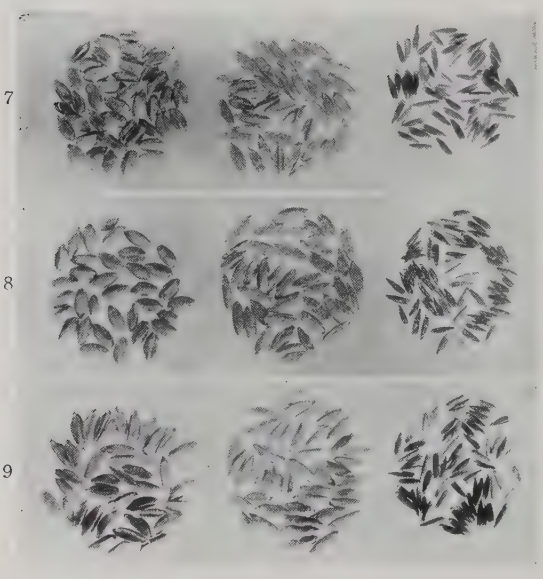


Photo. 7~8. Kernels of F_1 and its parents, from left to right: mother plant, F_1 and pollen plant. $\times$ca. $\frac{9}{10} \times \frac{3}{4}$.

7. *T. durum*, TdurSaF_1 and *S. africanum*.
8. *T. turgidum*, TturSaF_1 and *S. africanum*.
9. *T. dicoccum* var. *atratum*, TdicSaF_1 and *S. africanum*.

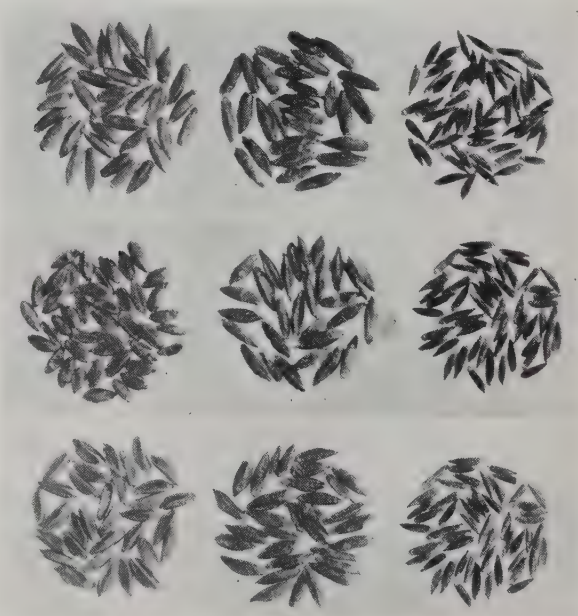


Photo. 10~12. Kernels of F_1 and its parents, from left to right: mother plant, F_1 and pollen plant. $\times$ca. $\frac{7}{10}$.

10. *T. polonicum*, TpolSmF_1 and *S. montanum*.
11. *T. pyramidale* Tpy. SmF_1 and *S. montanum*.
12. *T. dicoccum* var. *atratum*, TdicSmF_1 and *S. montanum*.

V. Seed fertility of F_1 plants

Every F_1 hybrid in the present research shows remarkably high percentages of fertility under natural selfing conditions. The results are shown in Table 4.

Table 4. Fertility of the F_1 and its parental plants.

Plants	Number of spikes	Number of spikelets	Number of kernels	Number of kernels per spike	Percentage of seed setting per spikelet	Weight of kernels per 1000 grains
T. durum	26	552	1420	54.59	257.25	42.00
T. turgidum	25	629	1269	50.74	201.75	34.00
T. polonicum	20	429	903	45.15	210.49	57.80
T. pyramidale	25	516	1320	52,80	255.81	41.90
T. dicoccum	20	581	892	44.60	153.53	43.00
S. africanum	10	504	302	30.19	59.92	6.80
S. montanum	20	892	950	47.50	106.50	6.60
$TdurSaF_1$	50	1814	1250	25.00	68.76	39.20
$TturSaF_1$	100	4118	717	7.17	17.41	39.50
$TdicSaF_1$	50	2043	1119	23.80	58.25	38.45
$TdurSmF_1$	25	930	3	0.24	0.32	
$TturSmF_1$	10	464	94	9.40	20.45	30.35
$TpolSmF_1$	120	4432	23	0.19	0.52	47.50
$TpySmF_1$	15	545	8	0.53	1.47	33.33
$TdicSmF_1$	20	809	133	6.65	16.44	38.50

As seen in Table 4, the seed fertility of $TdurSaF_1$ showed highest (68.76), and next came that of $TdicSaF_1$ (58.25), that of $TdurSmF_1$ being lowest (0.32). In general, the seed fertility of the F_1 hybrids between Emmer wheat and *S. africanum* was exceedingly higher than that of the F_1 with *S. montanum*.

A similar result concerning the seed fretility was also observed in the case of the hybrid between *T. persicum* and *S. africanum* ($TperSaF_1$) as well as between *T. persicum* and *S. montanum* ($TperSmF_1$). Furthermore, the seed fertility of $TperSaF_1$ was considerably higher than that of $TdurSaF_1$ (Nakajima 1956).

The average weight of F_2 seed obtained from every F_1 hybrid, lies closer to that of the mother plant than that of the intermediate between the parents (Table 4). The weight of 1000 grains of F_2 seed in each hybrid was as shown in Table 4. The F_2 seed usually had a wrinkled coat, while that of the parents have no wrinkles on the surface.

VI. Chromosome number of F_1 plants

The somatic number of chromosomes of the F_1 plants obtained from the eight combinations mentioned above, was 21 in every hybrid (Figs. 1~6). This

number exactly corresponds to the sum of the gametic number of the parents, viz., 14+7=21. In the meiosis of PMC's the same number of chromosomes was also observed as in the root tip cells.

Fig. 1~6. Somatic plates in root tip cells of F_1 plants between Emmer wheat and *Secale*. ×1200.
1. TdurSaF_1 $2n=21$.
2. TturSaF_1 $2n=21$.
3. TdicSaF_1 $2n=21$.
4. TdurSmF_1 $2n=21$.
5. TturSmF_1 $2n=21$.
6. TopolSmF_1 $2n=21$.

VII. Maturation division in PMC's

A. TdurSaF_1 plants

In the study of meiosis 11 of the 36 F_1 plants were selected at random. Of these, 4 have the number of bivalents 0~3 in PMC's at heterotypic metaphase; 6 have 0~4, the rest have 0~5. The frequency of bivalents in PMC's of the F_1 is shown in Table 5.

Table 5. Frequency of bivalents at IM of PMC's of TdurSaF_1 plants.

Individuals	Bivalents							
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	5_{II}	Mode (%)	Total
TdurSaF_1-1	424	201	60	14		1	0_{II} (60.57)	700
〃 2	266	132	79	23			0_{II} (53.20)	500
〃 3	363	179	51	7			0_{II} (60.50)	600
〃 4	380	161	45	14			0_{II} (63.33)	600
〃 5	298	210	71	18	3		0_{II} (49.67)	600
〃 6	408	142	37	11	2		0_{II} (68.00)	600
〃 7	365	198	66	19	2		0_{II} (56.15)	650
〃 8	447	183	59	9	2		0_{II} (63.86)	700
〃 9	346	239	86	23	6		0_{II} (49.43)	700
〃 10	401	173	59	17			0_{II} (61.69)	650
〃 11	394	181	85	33	7		0_{II} (56.29)	700
Total	4092	1999	698	188	22	1	0_{II}	7000
%	58.40	28.56	9.97	2.69	0.31	0.01	58.46	100.00

The case of zero in the number of bivalents was found to be the mode for each of 11 individuals.

The bivalents consist of two chromosomes of equal magnitude, and mostly they are stick-shaped, rarely ring-shaped ones were observed.

Usually, all univalents are scattered in the spindle at the metaphase of the heterotypic division as in *Triticum-Secale* F_1 (F_1-type division), but in the present case nearly all the univalents tend to lie on the equatorial plate. The percentage of the formation of the plate for total number of PMC's was shown in Table 6.

Table 6. Occurrence of the F_1-type division and the formation of equatorial plate at the heterotypic division in PMC's of TdurSaF_1 plants.

Individuals	F_1-type division		Formation of equatorial plate		Total (%)
	Number	%	Number	%	
TdurSaF_1-1	3404	99.01	34	0.99	3438 (100.00)
" 2	85	44.50	106	55.50	191 (100.00)
" 3	1740	99.83	3	0.17	1743 (100.00)
" 4	1674	78.74	452	21.26	2126 (100.00)
" 5	2224	100.00	0	0.00	2224 (100.00)
" 6	563	23.65	1818	76.35	2381 (100.00)
" 7	2530	98.37	42	1.65	2572 (100.00)
" 8	2145	89.78	244	10.22	2389 (100.00)
" 9	3416	88.54	441	11.46	3857 (100.00)
" 10	2270	92.35	188	7.65	2458 (100.00)
" 11	1659	82.83	344	17.17	2003 (100.00)
Total	21710	85.53	3672	14.47	25382 (100.00)

As shown in Table 6, PMC's that show the F_1-type division mixed with those that have univalents on the equatorial plate are usually observed, but sometimes PMC's showing the F_1-type division only or those having the equatorial plate alone are also observed.

To sum up, the formation of the equatorial plate is considerably less (14.47%) as compared with the F_1-type division (85.53%).

All individuals of this F_1 hybrid were fertile by natural selfing, and the fertility for the spikelet has shown to vary from 31.43 to 113.74% differing in different individuals, the average value being 68.76% (Table 4). The number of grains on a spike was found to vary from 7 to 49, the average number being 25.

B. TturSaF_1 plants

Among the 33 F_1 plants 10 were taken for cytological study. The number of bivalents found in one PMC at the hetrotypic metaphase was 0~4 in 4, and 0~5 in 6. The frequency of bivalents in one PMC is as shown in Table 7.

Figs. 7~15. Meiosis in PMC's of TdurSaF$_1$ hybrid. ×770.
7~14. heterotypic division. ×770.

7. 21 univalents scattered in spindle (TdurSaF$_1$-1).
8. Metaphase, side view, $1_{II}+19_I$ (TdurSaF$_1$-1).
9. do. $2_{II}+17_I$ (TdurSaF$_1$-1). 10. do. $3_{II}+15_I$ (TdurSaF$_1$-3).
11. do. $4_{II}+13_I$ (TdurSaF$_1$-7). 12. do. $5_{II}+11_I$ (TdurSaF$_1$-1).
13. Equatorial plate consisted only of univalents, side view.
14. do. polar view.
15. Homotypic metaphase, polar view, 2n plate with 21 chromosomes, one chromosome separated off (TdurSaF$_1$-1).

Figs. 16~23. Meiosis in PMC's of TturSaF_1 hybrid, heterotypic division. ×770.
16. 21 univalents scattered in spindle (TturSaF_1-1).
17. Metaphase, side view, $1_{II}+17_I$ (TturSaF_1-1).
18. do. $2_{II}+17_I$ (TturSaF_1-1). 19. do. $3_{II}+15_I$ (TturSaF_1-1).
20. $4_{II}+13_I$ (TturSaF_1-5). 21. do. $5_{II}+11_I$ (TturSaF_1-2).
22. Equatorial plate, consisted only of univalents, side view.
23. Form of bivalents and trivalents.
Figs. 24~31. Meiosis in PMC's TdicSaF_1 hybrids. ×770.
Figs. 24~29. Hetertypic division. ×770.
24. 21 univalents scattered in spindle (TdicSaF_1-1).
25. Metaphase, side view, $1_{II}\times19_I$ (TdicSaF_1-1).
26. do. $2_{II}+17_I$ (TdicSaF_1-1). 27. $3_{II}+15_I$ (TnicSaF_1-9).
28. Equatorial plate consisted only of univalents, side view.
29. do. polar view.
Figs. 30~31. Homotypic division. ×770.
30. Homotypic metaphase, 21 chromosomes. 31. do. 19:2 chromosomes.

Table 7. Frequency of bivalents at IM of PMC's of TturSaF_1 plants.

Individuals	Bivalents							
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	5_{II}	Mode (%)	Total
TturSaF_1-1	299	206	79	15	1		0_{II} (49.83)	600
" 2	276	198	89	27	8	2	0_{II} (46.00)	600
" 3	271	165	119	37	7	1	0_{II} (45.17)	600
" 4	313	191	73	18	5		0_{II} (52.17)	600
" 5	288	185	89	30	6	2	0_{II} (48.00)	600
" 6	183	198	136	66	16	1	1_{II} (33.00)	600
" 7	342	171	72	11	4		0_{II} (57.00)	600
" 8	373	146	55	21	5		0_{II} (62.17)	600
" 9	337	168	75	16	3	1	0_{II} (56.17)	600
" 10	774	490	228	77	28	3	0_{II} (48.38)	1600
Total	3456	2118	1015	318	83	10	0_{II}	7000
%	49.37	30.26	14.50	4.54	1.19	0.14	49.37	100.00

Among 9 individuals, 10 in all, configuration lacking bivalents appeared to be the mode, and in one case, one bivalent was the mode. The bivalents observed were generally stick-shaped, but sometimes, ring-shaped ones was also observed (Fig. 23).

Trivalents, V-shaped, were observed besides bivalents at the hetrotypic metaphase, but no tetravalent was found.

As to the behavior of the univalents, the F_1-type division was compared with the type in which univalents lie in the equatorial plate (Table 8).

Table 8. Occurrence of the F_1-type division and the formation of equatorial plate at the hetrotypic division in PMC's of $TturSaF_1$ plants.

Individuals	F_1-type division		Formation of equatorial plate		Total (%)
	Number	%	Number	%	
$TturSaF_1$-1	600	100.00	0	0.00	600 (100.00)
〃 2	600	100.00	0	0.00	600 (100.00)
〃 3	1533	82.02	336	7.98	1869 (100.00)
〃 4	896	88.89	112	11.11	1008 (100.00)
〃 5	1753	76.79	530	23.21	2283 (100.00)
〃 6	2755	94.12	172	5.88	2927 (100.00)
〃 7	2077	92.05	180	7.95	2257 (100.00)
〃 8	1093	73.45	395	26.55	1488 (100.00)
〃 9	1645	66.65	823	33.35	2468 (100.00)
〃 10	2503	85.52	412	14.48	2915 (100.00)
Total	15455	83.93	2960	16.07	18415 (100.00)

To sum up, the formation of the equatorial plate is by far less (16.07%) as compared with the F_1-type division (83.93%).

All individuals of this F_1 hybrid were fertile by natural selfing, and the fertility for the spikelet has shown to vary from 9.51 to 22.00% according to the individual, the average value being 17.41% (Table 4). The number of grains on a spike was found to vary from 1 to 20, the average number being 7.17.

C. $TdicSaF_1$ plants

In this combination, 11 F_1 plants were obtained and all of them were used in the cytological research. 0～3 bivalents were found in PMC's at the heterotypic metaphase in every plant. The frequency of bivalents in PMC's of the F_1 is as shown in Table 9.

The configuration lacking bivalents appeared to be the mode for each of 11 individuals. As in the former cases, most bivalents are stick-shaped. And in the present case there were no tri- and tetravalent.

In the heterotyic metaphase of PMC's the equatorial plate consisted only of the univalents was observed besides the F_1-type division as in $TdurSaF_1$ and $TturSaF_1$ plants. The percentage of the formation of the plate for total number of PMC's was shown in Table 10.

To sum up, the formation of the equatorial plate is considerably lesser (22.58%) than the F_1-type division (77.42%).

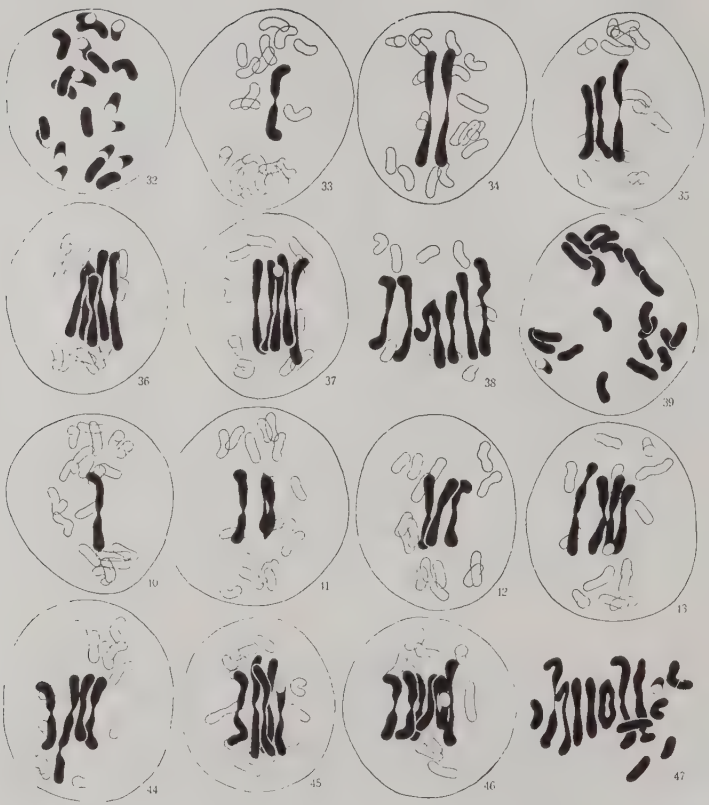
All individuals of this F_1 hybrid were fertile by natural selfing and the fertility for the spikelet was shown to vary from 37.94 to 79.27%, the average value being 58.25% (Table 4). The number of grains on a spike was found to vary from 10 to 45, the average number being 23.80.

Table 9. Frequency of bivalents at IM of PMC's of TdicSaF_1 plants.

Individuals	Bivalents					
	0_{II}	1_{II}	2_{II}	3_{II}	Mode (%)	Total
TdicSaF_1-1	280	187	31	2	0_{II} (56.00)	500
" 7	261	194	44	1	0_{II} (52.20)	500
" 9	278	192	29	1	0_{II} (55.60)	500
" 11	273	181	43	3	0_{II} (54.60)	500
" 12	291	168	38	3	0_{II} (58.20)	500
" 16	237	215	44	4	0_{II} (47.40)	500
" 17	237	224	33	6	0_{II} (47.40)	500
" 18	287	187	23	3	0_{II} (57.40)	500
" 19	225	217	53	5	0_{II} (45.00)	500
" 20	255	210	32	3	0_{II} (51.00)	500
" 21	229	217	46	8	0_{II} (45.80)	500
Total	2853	2192	416	39	0_{II}	5500
(%)	51.87	39.86	7.56	0.71	51.87	100.00

Table 10. Occurrence of the F_1-type division and the formation of equatorial plate at the heterotpyic division in PMC's of TdicSaF_1 plants.

Individuals	F_1-type division		Formation of equatorial plate		Total (%)
	Number	%	Number	%	
TdicSaF_1-1	2664	87.69	374	12.31	3038 (100.00)
" 7	1411	55.16	1147	44.84	2558 (100.00)
" 9	966	66.35	490	33.65	1456 (100.00)
" 11	1260	100.00	0	0.00	1260 (100.00)
" 12	1844	89.60	214	10.40	2058 (100.00)
" 16	1545	95.90	66	4.10	1611 (100.00)
" 17	1021	41.90	1416	58.10	2437 (100.00)
" 18	2278	91.38	215	8.62	2493 (100.00)
" 19	1339	60.45	876	39.55	2215 (100.00)
" 20	1864	89.44	220	10.56	2084 (100.00)
" 21	2071	87.02	309	12.98	2380 (100.00)
Total	18263	77.42	5327	22.58	23590 (100.00)



Figs. 32~38. Meiosis in PMC's of TdurSmF$_1$ hybrid, heterotypic division. ×770.
32. 21 univalents scattered in spindle (TdurSmF$_1$-8).
33. Metaphase, side view, $1_{II}+19_I$ (TdurSmF$_1$-8).
34. do. $2_{II}+17_I$ (TdurSmF$_1$-8). 35. do. $3_{II}+15_I$ (TdurSmF$_1$-8).
36. do. $1_{III}+3_{II}+12_I$ (TdurSmF$_1$-12).
37. do. $5_{II}+11_I$ (TdurSmF$_1$-12). 38. $1_{III}+5_{II}+8_I$ (TdurSmF$_1$-2).

Figs. 39~47. Meiosis in PMC's of TturSmF$_1$ hybrid, heterotypic division. ×770.
39. 21 univalents scattered in spindle (TturSmF$_1$-2).
40. Metaphase, side view, $1_{II}+19_I$ (TturSmF$_1$-2).
41. do. $2_{II}+17_I$ (TturSmF$_1$-2). 42. do. $3_{II}+15_I$ (TturSmF$_1$-2).
43. do. $4_{II}+13_I$ (TturSmF$_1$-2). 44. do. $1_{III}+3_{II}+12_I$ (TturSmF$_1$-2).
45. do. $5_{II}+11_I$ (TturSmF$_1$-4). 46. do. $6_{II}+9_I$ (TturSmF$_1$-2).
47. do. $7_{II}+7_I$ (TturSmF$_1$-9).

D. TdurSmF_1 plants

In the study of meiosis in PMC's, 8 individuals were used out of the total 36 F_1 plants obtained. In all the 8 individuals bivalents were observed within the limits of 0~5, while in 2 of them 6 bivalents were observed. The frequency of bivalents in PMC's of the F_1 is as shown in Table 11.

Table 11. Frequency of bivalents at IM of PMC's of TdurSmF_1 plants.

Individuls	Bivalents								
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	5_{II}	6_{II}	Mode (%)	Total
TdurSmF_1-2	117	98	148	96	28	10	3	2_{II} (29.60)	500
〃 3	74	74	89	48	13	2		2_{II} (29.67)	300
〃 4	143	85	145	91	31	5		2_{II} (29.00)	500
〃 5	153	127	126	65	22	7		0_{II} (30.60)	500
〃 6	147	179	152	80	32	9	1	1_{II} (29.83)	600
〃 7	173	192	139	68	24	4		1_{II} (32.00)	600
〃 8	224	143	84	39	7	3		0_{II} (44.80)	500
〃 12	141	121	134	64	32	8		0_{II} (28.20)	500
Total	1172	1019	1017	551	189	48	4	0_{II}	4000
%	29.30	25.48	25.43	13.78	4.73	1.20	0.10	29.30	100.02

As seen in Table 11, with regard to individual plants the mode of occurrence of bivalents was found to have the value 2 in 3 plants, 1 for other 2, and 0 for the remaining 3. When attention is paid to the whole 8 plants the configuration that lacks bivalents appeared to be the mode. Although stick-shaped bivalents were generally observed, and at most 2 ring-shaped ones were observed in one PMC. V-shaped trivalents were rarely observed besides bivalents, but no tetravalent was found at all.

The anthers of these F_1 plants seldom dehisced and the pollen grains were almost sterile, but sometimes some anthers dehisced and a few mature grains of seed were obtained by natural selfing.

E. TturSmF_1 plants

In this hybrid, 26 F_1 plants were raised, and 7 individuals were taken for cytological research. The number of bivalents found in one PMC at the heterotypic metaphase was 0~6 in 5 individuals, and 0~4 in one individual, and 0~7 in the remaining one. The frequency of bivalents in one PMC is as shown in Table 12.

As seen in Table 12, with regard to individual plants the mode of occurrence of bivalents was found to have the value 4 in one plant, 2 in 2, and 0 in the rest.

Table 12. Frequency of bivalents at IM of PMC's of TturSmF_1 plants.

Individuals	Bivalents									
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	5_{II}	6_{II}	7_{II}	Mode (%)	Total
TturSmF_1-2	207	147	192	92	44	12	6		0_{II} (29.57)	700
" 3	444	162	59	26	9				0_{II} (63.43)	700
" 4	194	122	237	141	78	23	5		2_{II} (29.63)	800
" 7	187	148	192	108	44	14	7		2_{II} (27.43)	700
" 8	248	213	153	55	24	5	2		0_{II} (35.43)	700
" 9	75	55	136	157	215	109	36	17	4_{II} (26.85)	800
" 10	202	148	145	77	20	6	2		0_{II} (33.67)	600
Total	1557	995	1114	656	434	169	58	17	0_{II}	5000
%	31.14	19.90	22.28	13.12	8.68	3.38	1.16	0.34	31.14	100.00

When attention is paid to the whole 7 plants the configuration that lacks bivalents appeared to be the mode. The bivalents were of normal type, and most of them were stick-shaped, though rare the ring-shaped ones were observed as in all the hybrids mentioned above. Though rare, trivalents, V-shaped and chain-shaped, were observed besides bivalents at the heterotypic metaphase, but no tetravalent was observed.

In most individuals of the F_1 plant used in this research, the meiosis of PMC's showed typical *Triticum-Secale* F_1-type division, but in one individual all the univalents were found to form the equatorial plate besides the F_1-type division (Table 13). This particular plant (TturSmF_1-3) alone showed fertility by natural selfing, though the percentage is not high, while other individuals were entirely sterile.

Table 13. Occurrence of the F_1-type division and the formation of equatorial plate at the heterotypic division in PMC's of TturSmF_1 plants.

Individuals	No. of preparation	F_1-type division		Formation of equatorial plate		Total (%)
		Number	%	Number	%	
TturSmF_1-3	a	133	46.67	152	53.33	285 (100.00)
	b	112	31.91	239	68.09	351 (100.00)
	c	134	96.40	3	3.60	137 (100.00)
Total		379	49.03	394	50.97	773 (100.00)

The fertility per spikelet has shown to vary from 8.89 to 34.15% according to the spikes in one individual, the average value being 20.45%. The number of grains on a spike was found to vary from 4 to 14, the average number being 9.4.

F. TpolSmF_1 plants

In the study of meiosis 12 individuals were used among 26 F_1 that have been obtained. Among the 12 individuals 6 have the bivalents 0～5 in PMC's at the heterotypic metaphase, and 5 ones have 0～4, the remaining one having 0～3. Frequency of bivalents in PMC's of the F_1 is shown in Table 14.

Table 14. Frequency of bivalents at IM of PMC's of TpolSmF_1 plants.

Individuals	Bivalents									
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	5_{II}	*R	**III	Mode (%)	Total
TpolSmF_1-1	375	147	40	6			3		0_{II} (66.02)	568
〃 2	174	167	130	66	12	1	25	3	0_{II} (31.64)	550
〃 3	188	141	148	52	18	3	9	1	0_{II} (34.18)	550
〃 4	386	186	75	14	3		9	2	0_{II} (57.88)	660
〃 6	209	192	108	34	7		11	1	0_{II} (38.00)	550
〃 7	185	162	130	67	14	2	12		0_{II} (33.04)	560
〃 8	324	186	41	8	1		5	1	0_{II} (57.86)	560
〃 9	199	183	106	45	15	2	18	1	0_{II} (36.19)	550
〃 12	215	202	167	85	18	3	18	7	0_{II} (31.16)	690
〃 27	224	179	114	28	5		14	1	0_{II} (40.73)	550
〃 28	348	159	36	6	1		10		0_{II} (63.27)	550
〃 30	258	158	90	34	9	1	6	2	0_{II} (46.91)	550
Total	3081	2062	1185	445	103	12	140	19	0_{II}	6888
%	44.73	29.94	17.20	6.46	1.49	0.17	2.03	0.28	44.73	99.99

* R denotes ring-shaped bivalents. ** III denotes trivalents.

The configuration lacking bivalents appeared to be the mode for each of 12 plants. Almost every bivalent consists of two elements of equal magnitude (normal bivalents), though exceptionally a few bivalents consisting of unequal elements (heteromorphic) were observed.

In rare cases, V- or chain-shaped trivalents were observed besides bivalents, but no tetravalent was found.

G. TpySmF_1 plants

In this combination, 3 F_1 plants were obtained, and they were all taken for cytological research. The number of bivalents found in one PMC at the heterotypic metaphase was 0～5 in 2 cases and 0～6 in the other. The frequency of bivalents in one PMC is as shown in Table 15.

As seen in Table 15, the mode of bivalents was found to be 2, 1 and 0 in the respective plants. In PMC's of the 3 plants taken together, the configuration lacking bivalents appeared to be the mode. Though sometimes, V-shaped trivalents and a N-shaped tetravalent were observed besides bivalents.

Figs. 48~53. Meiosis in PMC's of TpolSmF$_1$ hybrid, heterotypic division. ×770.
48. 21 univalents scattered in spindle (TpolSmF$_1$-2).
49. Metaphase, side view, $1_{II}+19_I$ (TpolSmF$_1$-2).
50. $2_{II}+17_I$ (TpolSmF$_1$-2). 51. do. $3_{II}+15_I$ (TpolSmF$_1$-2).
52. do. $4_{II}+13_I$ (TpolSmF$_1$-2). 53. do. $1_{III}+4_{II}+10_I$ (TpolSmF$_1$-2).
Figs. 54~63. Meiosis in PMC's of TpySmF$_1$ hybrid. ×770.
Figs. 54~62. Heterotypic division.
54. 21 univalents scattered in spindle (TpySmF$_1$-1).
55. Metaphase, side view, $1_{II}+19_I$ (TpySmF$_1$-1).
56. do. $2_{II}+17_I$ (TpySmF$_1$-1). 57. do. $3_{II}+15_I$ (TpySmF$_1$-1).
58. do. $4_{II}+13_I$ (TpySmF$_1$-1). 59. $1_{III}+3_{II}+12_I$ (TpySmF$_1$-1).
60. do. $1_{IV}+3_{II}+11_I$ (TpySmF$_1$-1).
61. do. $5_{II}+11_I$ (TpySmF$_1$-1). 62. do. $6_{II}+9_I$ (TpySmF$_1$-1).
63. Homotypic division, 14 chromosomes.

Table 15. Frequency of bivalents at IM of PMC's of TpySmF_1 plants.

Individuals	Bivalents										
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	5_{II}	6_{II}	*R	**III	Mode (%)	Total
TpySmF_1-1	128	124	147	130	54	6	1	9	1	2_{II} (24.92)	590
" 2	289	189	192	49	17	4		6		0_{II} (39.05)	740
" 3	298	312	243	81	12	4		29	1	1_{II} (32.84)	950
Total	715	625	582	260	83	14	1	44	2	0_{II}	2280
%	31.36	27.40	25.53	11.40	3.64	0.61	0.04	1.93	0.09	31.36	99.98

* R denotes ring-shaped bivalents. ** III denotes trivalents.

H. TdicSmF_1 plants

In this combination, 4 F_1 plants were obtained and they were employed in the cytological research, in which 3 have the number of bivalents 0~4 in PMC's at the heterotypic metaphase, and the rest having 0~3. The frequency of bivalents in PMC's of the F_1 is as shown in Table 16.

Table 16. Frequency of bivalents at IM of PMC's of TdicSmF_1 plants.

Individuals	Bivalents								
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	*R	**III	Mode (%)	Total
TdicSmF_1-1	810	789	293	70	9	31	2	0_{II} (41.10)	1971
" 2	552	721	270	73	12	22		1_{II} (44.23)	1630
" 3	304	379	43	4		9		1_{II} (51.92)	730
" 4	349	412	192	36	11	31	1	1_{II} (41.20)	1000
Total	2015	2301	798	185	32	93	3	1_{II}	5331
%	37.79	43.15	15.00	3.47	0.60	1.75	0.06	43.15	100.01

* R denotes ring-shaped bivalents. ** III denotes trivalents.

As seen in Table 16, the mode of bivalents was found to be 1 in 3 plants among the 4, and that of another one being zero. In the 4 plants taken as a whole, the configuration in which one bivalent appeared was the mode. Though of sometimes, V-shaped trivalents were observed in addition to bivalents; no tetravalent was found.

In the heterotypic metaphase of PMC's the equatorial plate consisting only of the univalents was observed besides the F_1-type division previously mentioned in TdurSaF_1, TturSaF_1, TdicSaF_1 and others. The percentage of the frequency of formation of the plate to total number of PMC's is as shown in Table 17.

As seen in Table 17, it was occasionally observed that in one anther PMC's that show F_1-type division were found together with those having the equatorial plate, while sometimes PMC's showing only the F_1-type division were observed. To sum up, the occurrence of the formation of the equatorial plate is considerably less frequent (37.69%), compared with the F_1-type division (62.31%).

Table 17. Occurrence of the F_1-type division and the formation of equatorial plate at the heterotypic division in PMC's of TdicSmF_1 plants.

Individuals	F_1-type division		Formation of equatorial plate		Total (%)
	Number	%	Number	%	
TdicSmF_1-1	2259	71.53	899	28.47	3158 (100.00)
" 2	1934	54.88	1590	45.12	3524 (100.00)
" 3	730	100.00	0	0.00	730 (100.00)
" 4	1264	50.20	1254	49.80	2518 (100.00)
Total	6187	62.31	3743	37.69	9930 (100.00)

Figs. 64~72. Meiosis in PMC's of TdicSmF_1 hybrid. ×770.
Figs. 64~71. Heterotypic division.
64. 21 univalents scattered in spindle (TdicSmF_1-1).
65. Metaphase, side view, $1_{II}+19_I$ (TdicSmF_1-1).
66. do. $2_{II}+17_I$ (TdicSmF_1-1). 67. do. $3_{II}+15_I$ (TdicSmF_1-1).
68. do. $4_{II}+13_I$ (TdicSmF_1-1).
69. Equatorial plate consisted only of univalents, side view (TdicSmF_1-1).
70. do. polar view (TdicSmF_1-1).
71. Metaphase, all univalents splitting longitudinally (TdicSmF_1-2).
72. Homotypic division, polar view of metaphase, 21 chromosomes.

I. The chromosome distribution at the heterotypic ana-telophase

The distribution of chromosomes to opposite poles at the ana-telophase in heterotypic division proceeded at random in F_1-type division, while in the case of the formation of equatorial plate some 21 chromosomes were distributed to each pole. In the former case proportions of 10 : 11~0 : 21 were observed. The frequency of distribution in the F_1 hybrids is given in Table 18.

Table 18. Distribution of chromosomes to the poles at the heterotypic ana-telophase in PMC's of F_1 hybrids.

Individuals	Distribution of chromosomes												
	0:21	1:20	2:19	3:18	4:17	5:16	6:15	7:14	8:13	9:12	10:11	Mode	Total
TdurSaF$_1$	105	10	8	20	12	18	19	10	20	31	69	0:21	322
%	32.61	3.11	2.49	6.21	3.73	5.59	5.90	3.11	6.21	9.63	21.43	32.61	100.02
TturSaF$_1$	27	10	9	19	28	43	39	33	61	61	143	10:11	473
%	5.71	2.11	1.90	4.02	5.92	9.09	8.24	6.98	12.90	12.90	30.23	30.23	100.00
TdicSaF$_1$	135	32	31	21	22	31	24	42	46	59	146	10:11	589
%	22.92	5.43	5.26	3.57	3.74	5.26	4.08	7.13	7.81	10.02	24.79	24.79	100.00
TdurSmF$_1$	35	14	9	1		6	16	26	35	99	202	10:11	443
%	7.90	3.16	2.03	0.23		1.35	3.61	5.87	7.90	22.35	45.60	45.60	100.00
TturSmF$_1$						1	3	7	11	30	103	10:11	155
%						0.65	1.94	4.52	7.10	19.35	66.45	66.45	100.01
TpolSmF$_1$				9	11	18	39	68	85	111	292	10:11	633
%				1.42	1.73	2.84	6.61	10.74	13.43	17.54	46.13	46.13	99.99

J. Tetrad stage

The number of cells consisting of a tetrad varied in the limits of 2~8 cells in most F_1 hybrids used in this research. The case of one consisted of 4 cells was shown to be the mode. The micronuclei resulting from irregular divisions were frequently observed in the cells of tetrads.

K. Summing up the data

Result of hybridization: The results of intergeneric hybridization carried out between 6 species of Emmer wheat and 2 species of *Secale*, either *africanum* and *montanum*, were as shown in Table 2 and Diagram A.

In a word, the seed fertility of the hybrids between the Emmer wheat and the *Secale* decreases in the following order: F_1 of *persicum*, *durum*, *turgidum*, *dicoccum* var. *atratum*, *polonicum* and *pyramidale* crossed with either *africanum* or *montanum*. In every hybrid, the seed fertility of F_1 obtained by the combinations with *montanum* was slightly higher than that of F_1 by those with *africanum*, excepting F_1 with *persicum*.

These values of seed fertility of the F_1 plants between Emmer wheat and *Secale* (*africanum*, *montanum*) are exceedingly higher than those of the F_1 between Emmer wheat and *cereale* or *Vavilovii*.

The affinity of hybridization seems stronger with wild *Secale* than with cultivated *Secale*.

Bivalents: At the heterotypic metaphase of PMC's of the F_1, of 12 combinations raised between the 6 species of Emmer wheat and of the 2 species of *Secale* the bivalents were commonly observed, 4 combinations of which were already published. The number of bivalents varied from 0~3 to 0~7 according to the combinations (Table 19).

Table 19. Frequency of bivalents at the heterotypic metaphase in meiosis of F_1 between 6 species of Emmer wheat and 2 species of *Secale*.

F_1	Bivalents										
	0_{II}	1_{II}	2_{II}	3_{II}	4_{II}	5_{II}	6_I	7_{II}	Mode	Mean	Total
TdurSaF_1	4092	1999	698	188	22	1			0_{II}	0.58	7000
%	58.46	28.65	9.97	2.69	0.31	0.01			58.46		100.00
TdurSmF_1	1172	1019	1017	551	189	48	4		0_{II}	1.43	4000
%	29.30	25.48	25.43	13.78	4.73	1.20	0.10		29.30		100.00
TturSaF_1	3456	2118	1015	318	83	10			0_{II}	0.78	7000
%	49.37	30.26	14.50	4.54	1.19	0.14			49.37		100.00
TturSmF_1	1557	995	1114	656	434	169	58	17	0_{II}	1.65	5000
%	31.14	19.90	22.28	13.12	8.68	3.38	1.16	0.34	31.14		100.00
TdicSaF_1	2853	2192	416	39					0_{II}	0.57	5500
%	51.87	39.86	7.56	0.71					51.87		100.00
TdicSmF_1	2015	2301	798	185	32				1_{II}	0.86	5331
%	37.79	43.15	15.00	3.47	0.60				43.15		100.01
*TpolSaF_1	3067	757	159	17					0_{II}	0.28	4000
%	76.68	18.93	3.98	0.43					76.68		100.02
TpolSmF_1	3081	2062	1185	445	103	12			0_{II}	1.01	6888
%	44.73	29.94	17.20	6.46	1.49	0.17			44.73		99.99
*TpySaF_1	2411	463	114	12					0_{II}	0.24	3000
%	80.37	15.43	3.80	0.40					80.37		100.00
TpySmF_1	715	625	582	260	83	14	1		0_{II}	1.31	2280
%	31.36	27.40	25.53	11.40	3.64	0.61	0.04		31.36		99.98
*TperSaF_1	3025	1852	981	268	24				0_{II}	0.77	6150
%	49.18	30.11	15.49	4.35	0.39				49.18		99.97
*TperSmF_1	1768	1068	1022	508	194	40			0_{II}	1.22	4600
%	38.43	23.22	22.22	11.04	4.22	0.87			38.43		100.00

* Already published.

Table 20. Formation of equatorial plate at the heterotypic division in PMC's of F_1 between 6 species of Emmer wheat and 2 species of *Secale*.

F_1	F_1-type division		Formation of equatorial plate		Total (%)
	Number	%	Number	%	
*TperSaF_1	8394	44.33	10543	55.67	18937 (100.00)
TturSmF_1	379	49.03	394	50.97	773 (100.00)
TdicSmF_1	5827	60.77	3761	39.23	9588 (100.00)
TdicSaF_1	18623	77.42	5327	22.58	23590 (100.00)
TturSaF_1	15455	83.93	2960	16.07	18415 (100.00)
*TpySaF_1	2595	84.64	472	15.39	3067 (100.03)
TdurSaF_1	21710	85.53	3672	14.47	35382 (100.00)

* Already published.

The number of bivalents varies not only with the mother plant, but also with the pollen parent. In other words, the variation may be said to have

taken place by the different influence caused by the different *Secale* used as the pollen parent on the chromosomes of the Emmer wheat as the mother plant.

In the F_1 of *montanum* the average number of bivalents and the order of dispersion of them surpass those of the F_1. This may be thought of as due to the autosyndesis between chromosomes of A and B genomes of Emmer wheat used as mother plant, as generally considered in the case of the F_1 between Emmer wheat and *S. cereale* by Kagawa and Chizaki (1934), Liljefors (1936), Aase (1930) and Nakajima (1950, 1951, 1953, 1955) and *T. dicoccoides* × *S. montanum* F_1 by Longley and Sando (1930) and others, also by Kihara (1936) in the haploid of *T. durum*.

The fact that the number of bivalents of the F_1 of *montanum* exceeds that of *africanum* seems due to the existence of some bivalents (bipartite) produced by conjugation of the chromosomes of *montanum* alone.

Formation of equatorial plate: In 7 F_1 among 12 combinations, the formation of equatorial plate was observed at heterotypic metaphase in PMC's (Table 20). 5 out of 7 F_1 were raised with *africanum* and two with *montanum*. The number of PMC's in which the equatorial plate was formed varied from 14.47 to 55.67 percent.

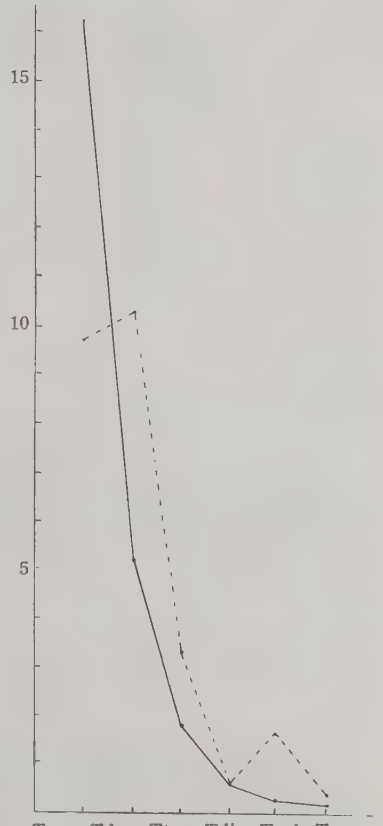


Diagram A. The percentage of F_1 plant obtained to the pollinated flowers.

In the F_1 in which the formation of equatorial plate was occasionally observed, bivalents are formed less frequently compared with the F_1 in which no equatorial plate is found. This fact seems due to the prevention of the formation of bivalents by forming the equatorial plate in the same stage.

Seed fertility of F_1 plants: Every F_1 plant of the 6 species of Emmer wheat and each of the 2 species of *Secale* was fertile by natural selfing, though not high, since they are intergeneric hybrids. The results were as shown in Table 4 and 21.

As seen in Table 21, the lower the mean value of the number of bivalents, and also the higher the percentage of the formation of equatorial plate, the

Table 21. Formation of equatorial plate at the heterotypic metaphase of PMC's and seed fertility of F_1 plants.

F_1	Average number of bivalents	% of formation of equatorial plate	Seed fertility	% of F_1 to pollinated flowers
$TperSaF_1$	0.77	55.67	94.88	16.05
$TdurSaF_1$	0.58	14.47	68.76	5.04
$TdicSaF_1$	0.57	22.25	58.25	0.52
$TpySaF_1$	0.24	15.39	45.38	0.19
$TturSmF_1$	1.65	50.97	20.45	3.41
$TturSaF_1$	0.78	16.07	17.41	1.85
$TdicSmF_1$	0.86	39.23	16.44	0.52
$TperSmF_1$	1.22		12.67	9.84
$TpolSaF_1$	0.28		1.69	0.33
$TpySmF_1$	1.31		1.47	0.36
$TpolSmF_1$	0.91		0.52	1.67
$TdurSmF_1$	1.43		0.32	10.34

higher the fertility, excepting one or two hybrids. This may be explained by considering that the formation of equatorial plate gives rise to the production of pollen that have 2n chromosomes more than those that have 2n/2 chromosomes; and the fertility goes roughly parallel with the rate of production of 2n pollen.

To sum up, the F_1 obtained with *africanum* showed higher fertility than with *montanum*. This seems to be due to the nature of pollen parents.

Summary

1) In the present investigation, cytological and genetical researches on the F_1 of eight combinations, between Emmer wheat (*durum, turgidum, dicoccum* var. *atratum, polonicum, pyramidale*) and *Secale* (*africanum, montanum*) were carried out.

2) The percentage of the F_1 plants to the number of the pollinated flowers were slightly higher in the combinations with *montanum* than that with *africanum*, excepting F_1 with *persicum* (Table 2).

3) The external characteristics of the F_1 plants resembled more closely the pollen parent than those that are intermediate, though they represent external characteristics of both parents (Table 3).

4) At the ripening stage, the spikelets of all F_1 plants become brittle, and this character may have been brought from the original characters of both *africanum* and *montanum*.

5) The F_1 plants were fertile in every combinations, though not highly fertile (Table 21).

6) The number of chromoromes, 2n, of F_1 plants of all combinations was

21 which corresponds to the sum of the gametic chromosome numbers of the parents.

7) The number of bivalents at heterotypic metaphase of PMC's varied from 0~3 to 0~7 according to the combinations (Table 19).

8) In the 7 F_1 hybrids, the formation of equatorial plate was observed at heterotypic metaphase in PMC's (Table 20), but the percentage of the formation varied from 14.47 to 55.67% according to the hybrids.

9) Every F_1 hybrids were fertile by natural selfing, though the percentages of fertility were not high (Table 4 and 21).

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Study of the Variegated Leaves, with Special Reference to Those Caused by Air Spaces.*

By

Noboru HARA

(Received August 14, 1956)

The term "variegated leaf" is used both in narrow and in wide senses. In the narrow sense it is applied to the leaves which have variegated parts caused by the deficiency of the chlorophyll, and corresponds to "panaschiertes Blatt" in German. In the wide sense, it is used to all variegated leaves, including the variegated leaves in the narrow sense, and corresponds to "buntes Blatt" in German (cf. Kōketsu 1914, Bateson 1919, Jackson 1953, Imai 1936). According to Schertz (1921), the term "mottled leaf" is used for the leaf which loses its chlorophyll in a diseased condition during its growing season.

There have been many morphological and anatomical studies on the variegated leaves in the narrow sense, which were summarized and discussed by Küster (1927).

The histogenetical analysis on the variegated leaves has been developed by subsequent studies and these studies have brought about available informations on the problem of leaf histogenesis (Thielke 1954). On the other hand, as to the variegated leaves in the wide sense, only a few studies have been carried out by Dalitzsch (1886), Hassack (1886), Stahl (1896), etc.

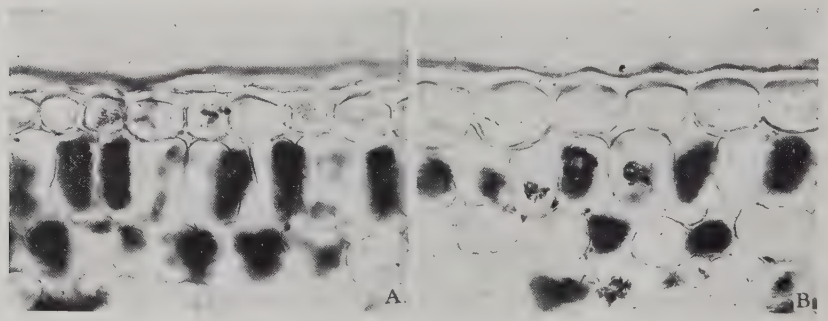

Fig. 1. *Heterotropa Takaoi* (×250): Photographs of cross sections of mature leaves. A. normal part, B. variegated part.

* Contributions from the Division of Plant Morphology, Botanical Institute, Faculty of Science, University of Tokyo, N.S. No. 78.

[Jap. Journ. Bot. **16**: 86-101. 1957]

In the present study, the variegated leaves in the wide sense are described, and especially the variegated leaves caused by diffuse reflection of light due to air spaces just beneath the epidermis are analysed from the developmental standpoint (Fig. 1).

Materials and Methods

Materials used in the present study are mostly taken from native and some cultivated plants in Japan. 55 species belonging to 24 families studied have

Table 1. Types of variegated leaves.

	A. Vein Type		B. Partial Type	C. Entire Type
	A 1. Advein Type	A 2. Intervein Type		
I. Chlorophyll Type	I • A1	I • A2	I • B	*
II. Air Space Type	II • A1	II • A2	II • B	II • C
III. Epidermis Type	*	*	III • B	*
IV. Pigment Type	IV • A1	IV • A2	IV • B	*

Asterisks show cases not to be found in this study.

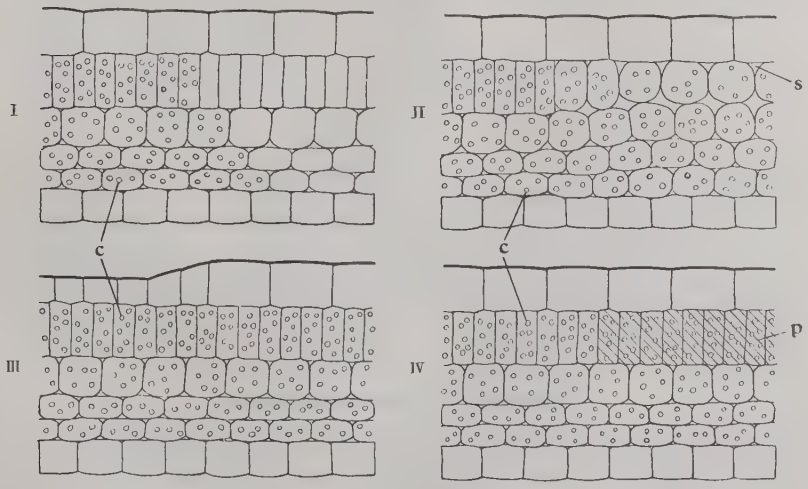

Fig. 2. Diagrams of types by causes of colours: I. Chlorophyll type, II. Air space type, III. Epidermis type, IV. Pigment type. c: chloroplast, s: air space, p: pigment.

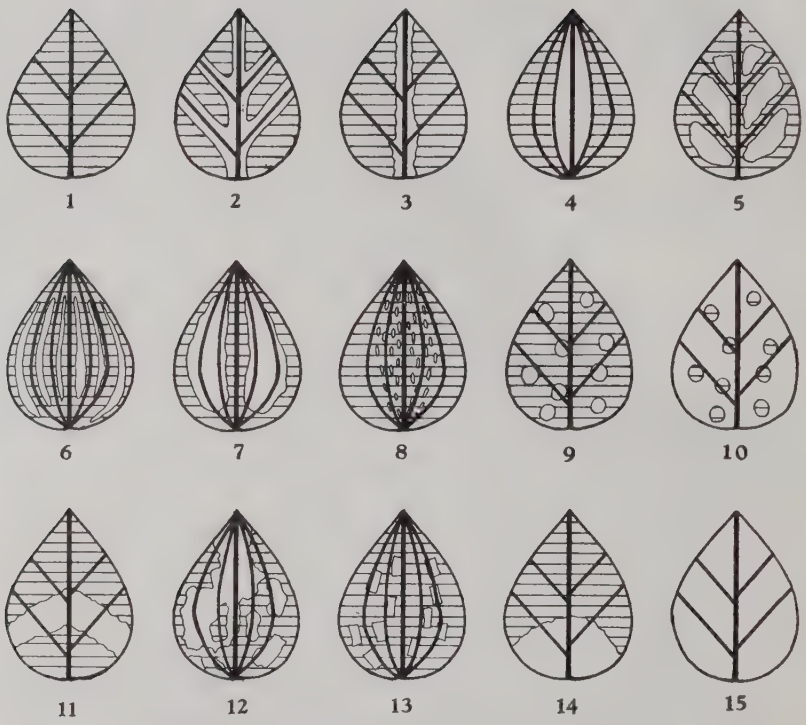

Fig. 3. Diagrams of types by positions: 1. Ordinary leaf, 2–4. Advein type, 5–7. Intervein type, 8–14. Partial type, 15. Entire type. Thick lines: veins, parts of fine lines: normal parts, parts of blank: variegated parts.

variegated leaves. The other species which had ordinary leaves were also used for the purpose of comparison with variegated ones.

Sections of some materials were observed without fixing and staining to distinguish the variegated part from the normal. Most of them were stained with haematoxilin, safranin, or light green, after fixing in FAA. Some quantitative treatments were used. Experiment of decapitulation was carried out on leaves of *Erythronium japonicum*.

Types of the variegated leaves

It is appropriate that the term "variegated leaf" is applied to the leaf which is partly or wholly coloured in other than ordinary green as their ordinary feature.

In this study four types of variegated leaves are tentatively recognized based on causes of colours in variegated parts, and these types are subdivided

by their positions, although Hassack recognized six types based on colours in variegated parts. These types are shown in Table 1, in which the marks "I–IV" denote causes of colours in variegated parts, and the marks "A–C" denote their positions. Combinations of both series of marks represent the types of variegated leaves.

Marks denoting the types:

Types	Causes of colours in variegated parts
I. Chlorophyll Type (Fig. 2: I)	Deficiency of chlorophyll.
II. Air Space Type (Fig. 2: II)	Existence of air spaces just beneath the epidermis.
III. Epidermis Type (Fig. 2: III)	Peculiarity of the epidermis.
IV. Pigment Type (Fig. 2: IV)	Existence of pigments other than chlorophyll.

Types	Positions of variegated parts
A. Vein Type (Fig. 3: 2–7)	Parts, related to vein or veins.
A1. Advein Type (Fig. 3: 2–4)	Vein and vein side parts.
A1a. (Fig. 3: 2)	Vein and vein side parts of all veins from major vein to minor veins.
A1b. (Fig. 3: 3)	Only vein and vein side parts of major vein.
A1c. (Fig. 3: 4)	Major vein part and intervein parts between major vein and a few minor veins beside it.
A2. Intervein Type (Fig. 3: 5–7)	Intervein parts.
B. Partial Type (Fig. 3: 8–14)	Parts, independent of veins.
C. Entire Type (Fig. 3: 15)	All parts.

Each type contains respectively the following species.

I. Chlorophyll Type (Fig. 2: I, Fig. 3)

I·A1a (Fig. 3: 2) *Cirsium Tanakae* Matsumura, *Silybum Marianum* Gaertn., *Goodyera Schlechtendaliana* Reichb., f.

I·A1b (Fig. 3: 3) *Zanthoxylum piperitum* DC., *Goodyera velutina* Maxim.

I·A1c (Fig. 3: 4) *Miscanthus sinensis* Anderss., *Crocus vernus* All.*

I·A2 (Fig. 3: 5) *Eupatorium chinense* L. var. *simplicifolium* Kitamura.

I·B *Actinidia polygama* Planch.[1], *Saururus chinensis* Baill.[1], *Goodyera hachijoensis* Yatabe (Fig. 3: 8).

II. Air Space Type (Fig. 2: II, Fig. 3)

II·A1a (Fig. 3: 2) *Chrysosplenium macrostemon* Maxim., *Cyclamen europaeum* L.[2], *Heterotropa japonica* F. Maekawa[3], *Heterotropa Takaoi* F. Maekawa, *Heterotropa tamaensis* F. Maekawa[3], *Pirola renifolia* Maxim., *Saxifraga stolonifera* Meerb., *Viola Tokubuchiana* Makino, *Viola Tokubu-*

1) cf. Fujita (1935). *Saururus chinensis*=*S. Loureirii* in the sense of Fujita.

2) Both II·A1a- and II·A2-types exist in the same leaf.

3) The other individuals with variegated leaves of the other type exist.

chiana Makino var. *Takedana* F. Maekawa f. *variegata* Makino.

II·A1b (Fig. 3 : 3) *Clematis apiifolia* DC., *Clematis Maximowicziana* Franch. et Sav., *Dumasia truncata* Sieb. et Zucc., *Heterotropa japonica* F. Maekawa[3)4)], *Heterotropa tamaensis* F. Maekawa[3)4)], *Mucuna capitata* Wight. et Arn., *Arisaema aequinoctiale* Nakai et F. Maekawa.

II·A1c (Fig. 3 : 4) *Tulipa latifolia* Makino, *Ornithogalum tenuifolium* Guss.

II·A2 (Fig. 3 : 5, The other cases are shown by figure numbers in round brackets) *Ainsliaea apiculata* Sch. Bip., *Akebia quinata* Decne., *Akebia trifoliata* Koidzumi, *Cyclamen europaeum* L.[2)], *Hepatica nobilis* Schreber var. *nipponica* Nakai f. *variegata* Nakai, *Heterotropa Blumei* F. Maekawa, *Heterotropa japonica* F. Maekawa[3)4)], *Heterotropa tamaensis* F. Maekawa[3)4)], *Schizophragma hydrangeoides* Sieb. et Zucc., *Peperomia Sandersii* DC. var. *argyreia* Hook. (Fig. 3 : 6), *Zebrina pendula* Schnizl. (Fig. 3 : 7).

II·B *Begonia argenteoguttata* Lemoine (Fig. 3 : 9), *Trifolium pratense* L. (Fig. 3 : 11), *Trifolium repens* L. (Fig. 3 : 11), *Erythronium japonicum* Decne.**, *Smilax Sieboldi* Miq. (Fig. 3 : 13), *Tricyrtis hirta* Hook.***, *Tricyrtis macropoda* Miq.***

II·C (Fig. 3 : 15) *Begonia Rex* Putz., *Chrysosplenium flagelliferum* Fr. Schm., *Circaea alpina* L.

III. Epidermis Type (Fig. 2 : III, Fig. 3)

III·B *Oxalis Martiana* Zucc. (Fig. 3 : 14)****.

IV. Pigment Type (Fig. 2 : IV, Fig. 3, Pigments are red in all cases ; Such tissues are shown in the brackets).

IV·A1b (Fig. 3 : 3) *Corylus Sieboldiana* Blume. [Upper epidermis][5)].

IV·A2 *Acer distylum* Sieb. et Zucc. (Fig. 3 : 5) [Mesophyll][5)], *Polygonatum lasianthum* Maxim. (Fig. 3 : 6) [Mesophyll].

IV·B *Pelargonium inquinans* Ait. (Fig. 3 : 11) [Mesophyll], *Polygonum*

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4) Both II·A1b- and II·A2-types exist in the same leaf.

5) The leaf of young plant has variegated parts.

* Mesophyll cells between epidermis and veins at variegated parts of the mature leaf have no chlorophyll, and become to the large lysigenous air spaces by their collapse after they expand extremely.

** The leaf forms its variegated parts of II·B-type compounding with IV·B-type. This variegated parts show nearly IV·C-type (Fig. 2: IV, Fig. 3: 15) in very young stages, but gradually become to the typical IV·B-type (Fig. 3: 12) losing their pigment. Moreover, the leaf in mature stage becomes to the compound variegated leaf with IV·B-type and II·B-type, forming air spaces just beneath the epidermis at parts where the pigments are lost.

*** The leaf forms its variegated parts of II·B-type compounding with IV·B-type. This variegated parts show nearly IV·B-type (Fig. 2: IV, Fig. 3: 9) in young stages, but gradually become to the compound variegated leaf with IV·B-type (Fig. 2: IV, Fig. 3: 9) and II·B-type (Fig. 2: II, Fig. 3: 10), forming air spaces just beneath the epidermis except the parts containing the pigment.

**** The ratio of the mean volume of epidermal cells in variegated parts to that in normal parts is 1.00 to 0.34.

longisetum de Bruyn (Fig. 3 : 11) [Upper epidermis], *Polygonum debile* Meisn. (Fig. 3 : 11) [Lower epidermis], *Polygonum filiforme* Thunb. (Fig. 3 : 11) [Upper epidermis], *Polygonum filiforme* Thunb. var. *smaragdinum* Ohwi (Fig. 3 : 11) [Upper epidermis]*****, *Erythronium japonicum* Decne. [Mesophyll]**, *Smilax China* L. (Fig. 3 : 13) [Mesophyll], *Tricyrtis hirta* Hook. [Mesophyll]***, *Tricyrtis macropoda* Miq. [Mesophyll]***.

Analysis of variegated leaves of air space type

The cause that variegated leaves of the air space type show silver white variegated parts is by the existence of the air in spaces just beneath the epidermis (conveniently, we call these air spaces as "air spaces of variegated parts"). In order to analize the reason why these air spaces are induced just beneath the epidermis, it is necessary to pursue the process of air space formation.

First of all, studies for the formation of schizogenous air spaces are reviewed. According to Sifton (1945), Duval-Jouve (1869, 1875) said that true lacunae were formed because the stretched cells could not keep pace with the growth of the surrounding layer. Sifton also pointed out that the schizogenous air spaces were formed because the middle lamella were pulled apart. Avery (1933), Tetley (1936), Cross (1940), Ryder (1954), etc. recognized that, because the growth of spongy cells could not keep pace with that of epidermal and palisade cells in development of the leaf, the strain was induced between spongy cells, thus forming air spaces between these cells. Thus, the force in plant body comes into question for the analysis of the air space formation. According to Matzke (1946), Harper (1916) recognized such factors as "mutual pressure, adhesion, surface tension, and the inherited form tendencies of the cells" in the algae as the underlying forces responsible for the actual forms of cells.

In the following parts, variegated leaves of the air space type are analyzed on the standpoint of developmental anatomy. Sinnott's method of quantitative analysis of histogenesis (Sinnott 1939), unit of which is the cell, is applied to the leaves of *Saxifraga stolonifera* and *Akebia trifoliata* so as to analyze the differential growth that may probably induce the strain. This method has been used in many studies after Sinnott has emphasized its necessity, but these studies have been applied hitherto only by the method measuring diameters of cells. Here, sectional areas of cells are measured by the planimeter for the purpose of understanding the growth volume at each stage of the development.

***** The ratio of the vertical length of palisade cells of variegated parts to that of normal parts is 1.0 to 0.8. Epidermal cells in variegated parts show helmet-like processes.

1. Advein type

A. *Saxifraga stolonifera*

a. Surface view of mature leaf: Variegated parts exist on vein parts and vein side parts (Fig. 4). Fig. 5 is the photograph of the surface view, showing the difference between variegated and normal parts.

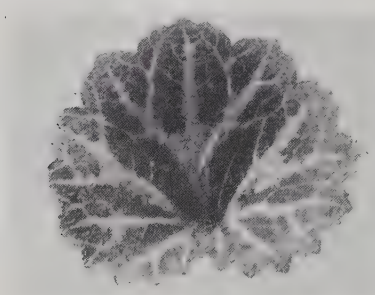

Fig. 4.

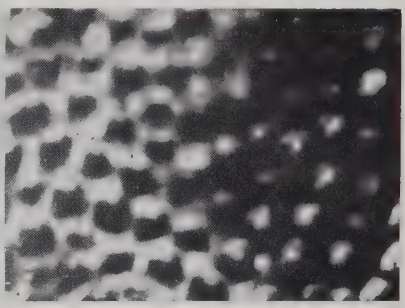

Fig. 5.

Fig. 4. *Saxifraga stolonifera* (×2): Photograph of surface view of mature leaf, showing variegated parts.

Fig. 5. *Saxifraga stolonifera* (×60): Photograph of surface view of mature leaf, showing part of boundary between normal and variegated parts.

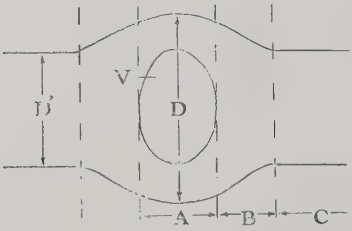

Fig. 6.

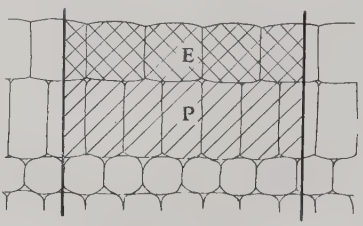

Fig. 7.

Fig. 6. Diagram of cross section of leaf: A. vein part, B. vein side part, C. intervein part, D. leaf thickness of vein part, D'. leaf thickness, V. vein.

Fig. 7. Diagram of cross section of leaf: E. sectional area of epidermis, P. sectional area of palisade tissue for E.

b. Observation on cross section of mature leaf (cf. Pl. 1: 1c, 2b): Leaf thickness at vein parts (cf. Fig. 6), especially at major vein parts, is naturally larger than the other parts. Air spaces between mesophyll cells at vein side parts are more abundant than at intervein parts. Air spaces between the epidermis and the palisade tissue also exist prominently in the former parts. Development of the palisade tissue at intervein parts are more remarkable than at the other parts.

c. Observation on development of leaf (cf. Pl. 1 : 1a, b, c): Eight leaves in different stages of development were cut off from plants at the same time, and successive cross sections of these leaves were made at the parts including the median major vein and vein side and intervein parts beside it, and the developmental process was pursued by means of comparison between these sections.

Ratios of the growth volume for each leaf were calculated as follows. In one section, successive ten cells of the epidermis at vein side and at intervein part respectively, as well as palisade tissue, are drawn by the Abbe's camera lucida. These treatment was carried out in two parts in one section. Sectional areas of these cells were measured by the planimeter, calculating the mean value per one cell of each part. On the other hand, numbers of palisade cells for successive ten epidermal cells of vein side part, as well as intervein part, were measured, calculating the mean value. Thus, the developmental transformation of ratios of the growth volume of the epidermis for the palisade tissue, which are expressed diagramatically as P/E in Fig. 7, could be shown as in Fig. 8 : a and b. The horizontal differential growth between the epidermis and the palisade tissue appears from the first stage of this treatment. After the stage of 19 mm leaf width, the growth of palisade tissue in intervein part keeps pace with the growth of the epidermis in this part still more, but not in vein side part. So, in the latter part, air spaces are formed between palisade cells and also between epidermal cells and palisade cells. On the other hand, the ratio, (Leaf thickness)/(Leaf thickness at vein part) is minimum at the stage of about 7 mm leaf width (Pl. 1 : 1b) as shown in Fig. 8 : c. The vertical growth of the vein is very rapid in the meristematic stages, and mesophyll cells surrounding the vein also grow more rapidly than mesophyll cells of the other parts, resulting their earlier maturation. Because the vertical growth of mesophyll cells surrounding the vein cannot keep pace with the

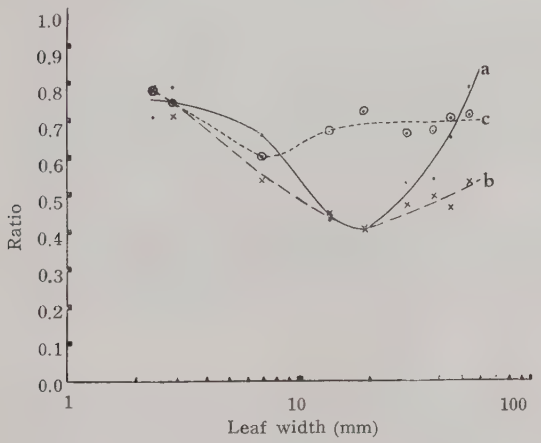

Fig. 8. *Saxifraga stolonifera :* a. the change of the ratio, (sectional area of palisade tissue)/(sectional area of epidermis) in normal part (intervein part) for leaf width, b. the change of the same ratio in variegated part (vein side part) for leaf width, c. the change of the ratio, (leaf thickness) / (leaf thickness of vein part) for leaf width.

vein in the later stages, air spaces in vein side part are more abundant than in intervein part.

Thus, the two differential growth, horizontal and vertical, appear first of all in the leaf development, and air spaces appear secondarily. The first stage in which variegated parts are seen with the naked eyes is attained when the leaf grows to about 13.5 mm in leaf width. The numbers of layers in the mesophyll increase gradually, but those in the palisade tissue do not increase.

d. Comparison with ordinary leaves of related species (cf. Pl. 1: 2a, b, c): Mature leaves of *Saxifraga stolonifera* was compared with mature leaves of *Saxifraga Fortunei* Hook. var. *incisolobata* Nakai and of *Tanakaea radicans* Franch. et Sav.

The following results for the leaves of *Tanakaea radicans* and *Saxifraga stolonifera* was obtained statistically by means of sectional area measurement of cells as explained before:

(Mean sectional area of palisade cells at upper part of vein)
$<$(Mean sectional area of palisade cells at vein side part)
$<$(Mean sectional area of palisade cells at intervein part)

These relations cannot be recognized positively in the case of *Saxifraga Fortunei*, although there are more or less such tendencies. On the other hand, ratios, (leaf thickness)/(leaf thickness at vein part) for the leaves of *Tanakaea radicans*, *Saxifraga stolonifera*, and *S. Fortunei* are 0.51, 0.71, and 0.89, respectively.

From these facts, we can recognize that the growth of the vein in the leaf affects to the growth of the neighbouring palisade cells. The marked difference between the leaf of *Tanakaea radicans* and of *Saxifraga stolonifera* is that the former has not the air spaces just beneath the epidermis.

B. *Heterotropa*

There are three types in the leaves of *Heterotropa*, and both variegated parts of the advein type and those of the intervein type exist in one leaf in some cases.

It is observed that mesophyll cells surrounding the vein do not cohere closely with the neighbouring cells in the case of the variegated leaf, while these cells of the ordinary leaf cohere more closely with the neighbouring cells.

C. Others

Ordinary leaves of *Viola grypoceras* A. Gray was compared with variegated leaves of *Viola Tokubuchiana* and *V. Tokubuchiana* f. *variegata*. Only difference between the ordinary leaf and the variegated one is in the point that there are remarkable air spaces just beneath the epidermis of vein and vein side parts in the case of the variegated leaf.

In the leaves of *Tulipa latifolia* and *Ornithogalum tenuifolium*, large air spaces just beneath the epidermis and between mesophyll cells, hugeness of mesophyll cells, low density of chloroplasts, and collapse of mesophyll cells are observed at variegated parts.

D. Discussion on the advein type

It is certain that there is the horizontal strain between palisade cells, which is induced by the differential growth between the epidermis and the palisade tissue in the leaf, especially in variegated parts of the variegated leaf. This horizontal strain is the important factor to the air space formation of variegated parts just above the veins.

On the other hand, it is also certain that one of the other factors for the variegated leaf formation is the vertical strain induced by the vertical growth of the vein. Due to the rapid vertical growth of the vein in the meristematic stages, mesophyll cells surrounding it grow rapidly, resulting their earlier maturation than mesophyll cells of intervein part and in formation of large air spaces between these cells. This observation supports Eames's opinion (1951) that the cells about veins cease their cell division earlier than the other parts.

These strains, horizontal and vertical, are observed in the ordinary leaves as well as the variegated leaves, so that, these strains are not characteristic of the variegated leaves. There must be the other important factor, that is, it must be considered that air spaces of variegated parts are formed by the plastic character of middle lamella between cells which apt to be separated one another easily by the strains.

Physical factors, horizontal and vertical, and perhaps some chemical factors have their influence on the leaves of various species to form the various degrees of variegation in the advein type.

It can be thought in the case of the leaves of *Tulipa* and *Ornithogalum* that there may be some factors which prompts abnormally the growth of cells at the variegated parts.

2. Intervein type

A. *Akebia trifoliata*

a. Surface view of mature leaf: Variegated parts exist on intervein parts (Fig. 9).

b. Observation on cross section of mature leaf (cf. Pl. 1: 3b): Differentiation of the palisade tissue is not clear in variegated parts, and there are air spaces just beneath the epidermis and between palisade cells.

c. Observation on development of leaf (cf. Pl. 1: 3a, b): As in the case of *Saxifraga*, growth ratios between the epidermis and the palisade tissue are measured both at variegated and normal parts. Seven leaves in different stages were cut off from plants at the same time. Future variegated and normal parts were decided by presumption for young leaves in which variegated parts was not obvious for naked eyes. But even these parts could not decided for very young leaves of 1.3 mm and 2.1 mm leaflet width. Growth of the palisade tissue which will become to variegated parts in future does not keep pace with growth of the epidermis after the certain stage, although it shows active development in very young stages (Fig. 10). Spongy tissues

Fig. 9. *Akebia trifoliata* (×1): Photograph of surface view of mature leaf, showing variegated parts.

are not different at both parts, regardless of difference in palisade tissues. Leaf thickness and numbers of mesophyll layers were constant from the first stage to the last in this study, excepting vein parts, which grow more or less up and down and emerge from the lamina resulting no influence to the growth of the mesophyli, so that, the leaf exclusively shows the plane expansion. The stage in which variegated parts are seen with the naked eyes is about 20.0 mm of leaflet width.

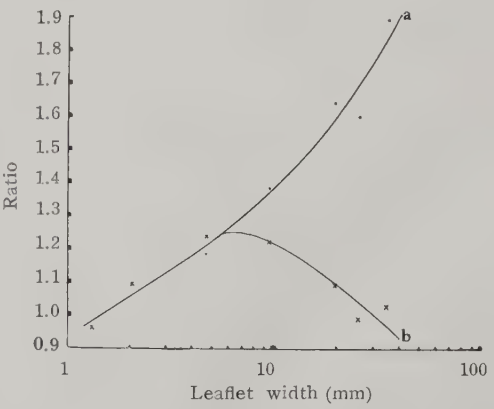

Fig. 10. *Akebia trifoliata:* a. the change of the ratio, (sectional area of palisade tissue)/(sectional area of epidermis) in normal part for leaflet width, b. the change of the same ratio in variegated part for leaflet width.

The leaf of *Akebia trifoliata* becomes not always variegated. Air spaces which correspond to air

spaces of variegated parts are not formed in the case of the non-variegated leaf.

B. Others

The leaf of *Ainsliaea apiculata* resembles the leaf of *Akebia*. The leaves of *Cyclamen*, *Heterotropa* etc. resemble also the leaf of *Akebia*, although leaf thickness and numbers of layers in the mesophyll increase more or less remarkably.

C. Discussion on the intervein type

It is thought that air spaces of variegated part of the intervein type are formed by the horizontal strain due to the differential growth between the epidermis and the subepidermal layers, and also by the plastic character of the middle lamella.

3. Partial type

A. *Erythronium japonicum*

a. Surface view and observation on section of leaf: The leaf of this species has its prominent leaf tip (Fig. 11). The upper side of mature leaf is coloured in silver white, green, and brown confusedly, although it is coloured almost in brown in its young stages. The parts between silver white and

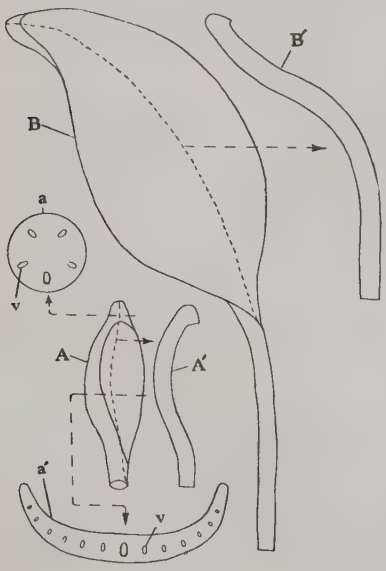

Fig. 11.

Fig. 11. Diagrams of leaf of *Erythronium japonicum*: A. young leaf, B. mature leaf, A'. median longitudinal section of A, a. cross section of tip of A, a'. median cross section of A, B'. median longitudinal section of B, v. veins.

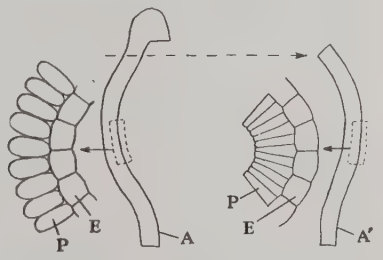

Fig. 12. Diagrams of young leaf of *Erythronium japonicum*: A. median longitudinal section of young involute leaf, A'. median longitudinal section of young leaf, when leaf tip was cut off. E. epidermis, P. palisade tissue.

brown parts are always green, and brown parts alter to silver white after they alter to green developmentally. There are air spaces just beneath the epidermis at silver white parts, and the pigment in mesophyll cells at brown parts. The young leaf is involute (Fig. 11: A), and the mature leaf is somewhat revolute (Fig. 11: B).

b. Experiment and its result: When the leaf tip is cut off in a young stage (about 4.5 cm length of the lamina), the change that the involute state alters into the revolute is promoted remarkably, and the changes that brown parts alter into green and that green parts alter into silver white are delayed remarkably. But, this treatment alters only the process of leaf development, for the leaf size, their state, and their colours become to be almost same in both treated and untreated mature leaves.

c. Discussion on variegated leaf of *Erythronium japonicum:* As shown longitudinally in Fig. 12, as well as transversally, it is certain that epidermal cells in the involute state are more compact and subepidermal layer cells are more loose than in the revolute state. The horizontal strain is apt to be induced between subepidermal layer cells and air spaces are apt to be formed between these cells in the former state. It is contrariwise in the latter state. In natural state, it is obvious that air spaces of variegated parts in the young leaf are formed by the strain, induced by the differential growth between the epidermis and the subepidermal layer, and by influence of the leaf tip, which supports the lamina in the involute state mechanically in the young stages, for these possibilities are certainly expected by the result of the treatment mentioned above. It is expected that these air spaces in the later stages, in which the greater part of the lamina is under no mechanical influence of the leaf tip for expansion of the lamina, are formed only by the strain induced by the differential growth.

It is necessary to consider about the relation between the air space formation and the pigment. Air spaces of variegated parts are formed after the pigment is lost in these parts of the leaf of *Erythronium.* In the variegated leaf of *Tricyrtis,* air spaces between subepidermal layer cells are formed avoiding the parts which have the pigment. In the variegated leaf of *Polygonum filiforme* var. *smaragdinum,* the length of palisade cells in variegated parts which have the pigment in the epidermis is longer than in normal parts which have no pigment in the epidermis. Thus, it is recognizable that the pigment has very intimate relationships to development of the mesophyll and the air space.

B. Others

The round variegated part of the leaf of *Begonia argenteoguttata* has a hair on its center. The formation of this variegated parts is thought to be related with the hair growth.

In the variegated leaves of *Trifolium pratense, T. repens,* and *Smilax Sieboldi,* there are remarkable deficiency of the chlorophyll in mesophyll cells as well as the air space formation of variegated parts. So, it can be

thought that there are perhaps some factors which inhibit the cell growth itself.

White meandering strands are seen often on the leaf surface by parasites in leaf tissue (for example, on the leaf surface of *Ranunculus* and *Hosta*). This phenomenon occurs because the air substitutes for palisade cells or mesophyll cells after they are eaten by parasites. The leaf which has variegated parts caused by some parasites or deseases is not the "variegated leaf", because this feature is not the ordinary one of this leaf.

Conclusion

The table of the types of variegated leaves which is based on causes of colours of variegated parts and on their positions is tentatively set up for description. But, it is appropriate that we recognize four types based on causes of colours, for these causes are characteristic respectively. And also it is certain that causes of the formation of variegated parts are related intimately with their positions of the air space type.

Air spaces exist more or less just beneath the epidermis of the ordinary leaf. But, only when these air spaces are abundant extremely, we can recognize variegated parts on the surface of these leaves.

Following views are possible from the analysis of variegated leaves of the air space type. There is generally more or less the differential growth between cells or tissues, which are developing, in plant body. These differential growth induce the stress and strain between cells or tissues. These stress and strain give influence more or less to the differentiation of cells and tissues. We can recognize that there are generally two strains acting on mesophyll cells in process of the leaf development. One is the horizontal strain induced between mesophyll cells by the differential growth between the horizontal growth of the epidermis and of the mesophyll. Another is the vertical strain induced between mesophyll cells of the vein side part by the differential growth between the vertical growth of the vein part and of the vein side part.

When these strains act to separate the middle lamella between epidermal and subepidermal cells, and at the same time the middle lamella in this part has the plastic character which is apt to be separated by strains, this leaf develops to a variegated leaf of the air space type. The horizontal strain acts on the vaiegated leaf formation of the intervein type, the partial type such as *Erythronium*, and perhaps of the entire type. The vertical strain, and also the horizontal strain in the most instances, act on the variegated leaf of the advein type. Some other factors which regulate the growth of mesophyll cells in variegated parts are expected in the leaves of *Tulipa*, *Ornithogalum*, *Trifolium*, and *Smilax* in the air space type.

Most variegated leaves of the air space type are the leaves of shade plants, palisade tissue of which is more inactive than of sun plants. This fact confirms the conclusion, based on the strains, as to the variegated leaf formation

of the air space type, because it can be thought that the differential growth between the epidermis and the palisade tissue is apt to be induced by the inactive growth of the palisade tissue.

Stomata do not affect to the origination of air spaces, because the differentiation of stomata usually occurs after intercellular spaces, which will become to air spaces, originate. It will be easily suspected that stomata affect only to facilitate ventilation of the air in leaf tissues and also stomata have only this influence for the air space formation of the variegated parts.

Summary

Types of variegated leaves in the wide sense are divided by causes of colours in variegated parts as follows; I. Chlorophyll type, II. Air space type, III. Epidermis type, and IV. Pigment type. These types are subdivided by positions of variegated parts respectively as follows; A. Vein type (A1. Advein type, and A2. Intervein type), B. Partial type, and C. Entire type.

It is certain in the most variegated leaves of the air space type that air spaces just beneath the epidermis in variegated parts are formed mainly by the following factors; 1) Horizontal strain, which is induced by the differential growth between the epidermis and the palisade tissue, 2) Vertical strain, which is induced by the differential growth between vein part and vein side part, and 3) Plastic character of the middle lamella, by which the cells are to be separated one another by the strains.

The author wishes to express his best thanks to Prof. Em. Y. Ogura for his kind direction, and also to Dr. S. Watari for his valuable advices.

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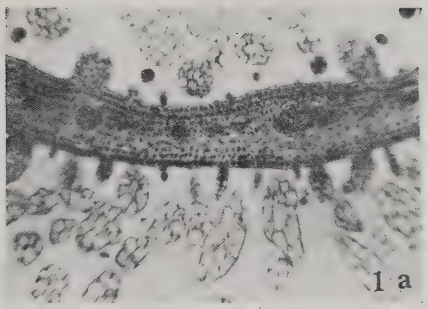
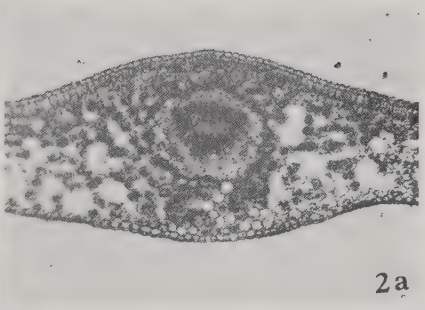
Explanation of Plate I.

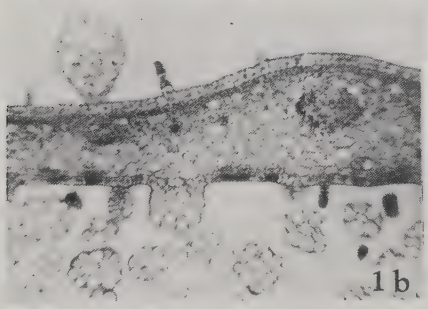
Fig. 1. a~c. *Saxifraga stolonifera* (×100): Cross sections of leaves. a. very young leaf (leaf width: 2.4 mm), b. (6.9 mm), c. somewhat matured leaf (45.0 mm).

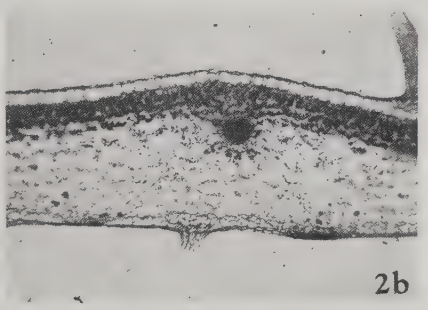
Fig. 2. a~c. Cross sections of mature leaves: a. *Tanakaea radicans* (×45), b. *Saxifraga stolonifera* (×30), c. *Saxifraga Fortunei* (×30).

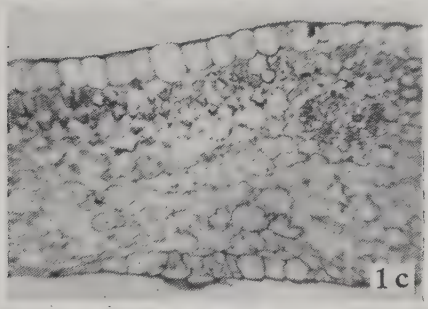
Fig. 3. a, bN, bV. *Akebia trifoliata* (×440): Cross sections of leaflet. a. very young leaf (leaflet width: 2.1 mm), b. somewhat matured leaf (35.0 mm), bN. normal part, bV. variegated part.

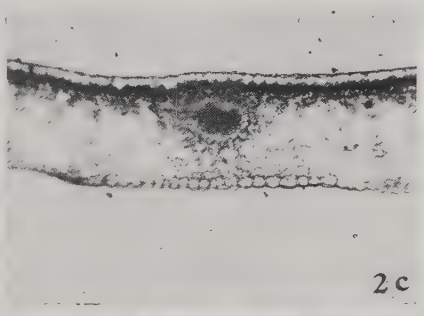


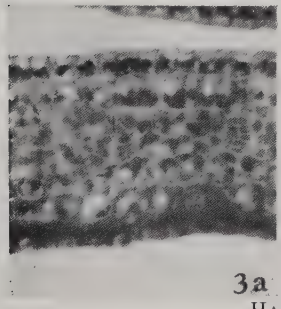


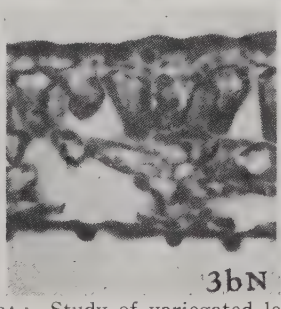


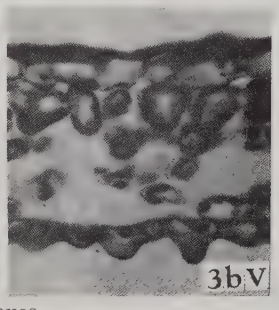












HARA: Study of variegated leaves

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Pollen Analytical Studies on Bogs of Central Japan, with Special References to the Climatic Changes in the Alluvial Age.

By

Shoichi HORI

(Received August 15, 1956)

1. Introduction

For the elucidation of the climatic changes in the alluvial age the study of the advance and retreat of glaciers has been regarded, especially in northern Europe, as one of the most reliable methods. Hence the examinations of glacial land forms and of glacial deposits such as glacial varve clay have been extensively made in Europe with valuable results. In Japan, however, these methods are hardly applicable, since the remains of glacial valleys are generally poor and imperfect, and the existence of glacial varve clay remains doubtful.

On the other hand the pollen analytical procedure has advantages for the estimation of the past climatic changes of the central part of Japan in that in its hill districts there are several high bogs containing well preserved pollen grains, from the nature of which the forest succession can be traced without difficulty.

Consequently, especially in Japan, the pollen analytical method seemed to be the most suitable one for the study of climatic changes in the alluvial age.

Since 1935 the writer has conducted pollen analytical researches upon bogs in the central part of Japan, and obtained some results which might be able to contribute to the knowledge of the forest successsion and the climatic changes.

Before going further, the writer wishes to express his cordial thanks to Professor Tomoo Miwa of the Tokyo University of Education, for his encouragement as well as for many valuable suggestions given throughout the whole course of this work.

2. Historical Review

a. Pollen Analyses in Japan

Concerning the pollen analytical studies in Japan, there are a number of important researches, namely Nakano (1933) upon Ozegahara; Miyai (1933, 1935, 1938) upon Usagishima in Nikko, Yakushima and Mt. Kirishima; Numata and Tamada (1936, 1939) upon Kyoto and its vicinity, Mt. Ontake and Tateshina; Jimbo (1936) and Nakamura (1942) upon Mt. Hakkoda, Matsushima (1941) upon

[Jap. Journ. Bot. **16**: 102–127. 1957.]

Korea; and Yamazaki (1937, 1942, 1943) upon Saghalien and Hokkaido.

With the exception of the works by Numata and Tamada, and by Yamazaki, pollen analyses were conducted with relatively thin peat layers of only about 1 m depth. Moreover, the results of these workers were not conclusive but

Fig. 1. Bogs in Central Japan: (1) Yashimaga-hara, (2) Odoriba, (3) Karuizawa, (4) Asama-yama, (5) Sugadaira, (6) Yoshiga-daira, (7) Nozori-ike, (8) Naeba-yama, (9) Oomine-numa, (10) Kakuman-buchi, Akagi-yama, (11) Kobuga-daira, (12) Usagi-shima, (13) Kinu-numa, (14) Ozega-hara, (15) Sanpooji-ike; all these bogs were studied by the writer.

remained fragmentary. Numata and Tamada (1936, 1939) from their studies at Ontake, Tateshina and Yashimaga-hara (peat layer 3.7 m in depth) divided the forest succession into five periods, from bottom to top layer, as follows: 1. *Pinus-Picea-Tsuga* period, 2. *Quercus-Fagus* period, *Alnus-Betula* period, 4. *Quercus-Fagus* period, 3. *Alnus-Betula* period, 4. *Quercus* period, 5. *Pinus* period. But they did not treat the problem of climatic changes.

Yamazaki (1937, 1942, 1943), from his studies in Hokkaido and Saghalien, decided the climatic changes from the deluvium to the alluvium period in northern part of Japan. According to his results there was in nothern Japan no warm but only cold period.

b. Studies by the Writer

The writer has performed pollen analytical studies since 1935 upon bogs in the central part of Japan, such as Yashimaga-hara (1938), Odoriba (1940), Sanpooji-ike (1941 a), Ozega-hara (1941 b), Naeba-yama, Asama-yama, Yahazu-daira, Yoshiga-daira, Akagi-Kakumanbuchi, Sugadaira (1949 a), Nozori-ike and Oomine-numa (1949 c) (Fig. 1).

Among them, the bogs of Yashimaga-hara and Oomine-numa contain peat layers more than 8 m in depth, the deepest in the central part of Japan, from which samples were collected and pollen analytical researches performed. These two bogs are situated at the lower part of the coniferous forest zone and simultaneously at the upper part of the deciduous forest zone, where climatic changes exert direct influences on the forest composition and are reflected upon the pollens contained in the peat. Chiefly about the peat layers of these bogs, the writer performed the studies by Erdtman's method (1931, 1933, 1934) which is considered best.

In Northern Europe and North America, the number of years required for peat layer deposition was determined by the age of glacier deposits, by the annual ring of trees and also by relics of ancient people buried in peat layers. In Japan these materials are completely wanting. Therefore, in order to determine the age of climatic changes which can be seen from pollen analyses, the writer made use of volcanic ashes imbedded in the peat layer. By examining the properties of the volcanic ash microscopically and the age of the eruption by the literature, the chronological scale of the rate of peat growth and of the climatic changes in the Alluvial age could be obtained.

3. Materials and Method

In order to obtain the materials for the research, the Swedish sampler was used; peat was obtained in a cylinder from, and from each layer 1 cc. of the sample was taken and treated as follows:

Erdtman's method mentioned above: A mixture of acetic anhydride and concentrated sulphuric acid in a ratio of 9 : 1 was added to about 1 g of peat, and after heating several minutes in a water bath, water was added and well stirred. By means of a centrifuge the sediment was collected and washed with

water several times, sealed in glycerol jelly, and 175~200 pollens of each sample were identified under microscope.

The writer, however, found it preferable to allow the pollen to sediment by itself in place of centrifugation, for the pollens of *Pinus*, *Picea* and *Abies* are liable to be destroyed by the use of a centrifuge.

4. Results

A) Results of Pollen Analyses

The writer (1938, 1940, 1941 a, 1941 b, 1949a, 1949 b, 1949 c) performed pollen analyses upon several bogs in the central part of Japan. Among them the bogs of Yashimaga-hara and Oomine-numa have extraordinarily thick peat layers (over 8 m) and the results from them are considered most significant. Therefore they are given here in detail and those from other bogs briefly only for comparison.

a) Yashimaga-hara Bog

Yashimaga-hara bog is situated about 8 km northeast of Kami-suwa, between Kuruma-yama (1925 m) and Washiga-mine (1798 m) in Nagano prefecture. This high bog, about 0.25 square kilo meters in area, rises in its eastern and western parts; the former has 805 cm peat layer, and the latter 710 cm one. The samples of the peat were taken on the eastern hillock.

From the peat layers, various species of pollens were found; but among them pollens of 12 species such as *Pinus*, *Abies*, *Tsuga*, *Quercus*, *Fagus*, *Corylus*, *Betula*, *Alnus* *Ulmus*, *Picea*, *Juglans* and *Pterocarya* were chosen to obtain percentages of appearance and to build the pollen diagram (Fig. 2).

The percentage of *Pinus* pollen is above 70% near the uppermost layer, decreases gradually downward, and becomes lower than 10% from 230 to 380 cm. It increases a little from 390 to 470 cm, decreases again with the increases of depth, and then rises once more near the bottom, reaching above 20%.

The percentage of *Abies* is generally low. It is somewhat high (13%) at 300 cm, becoming above 20% at the bottom.

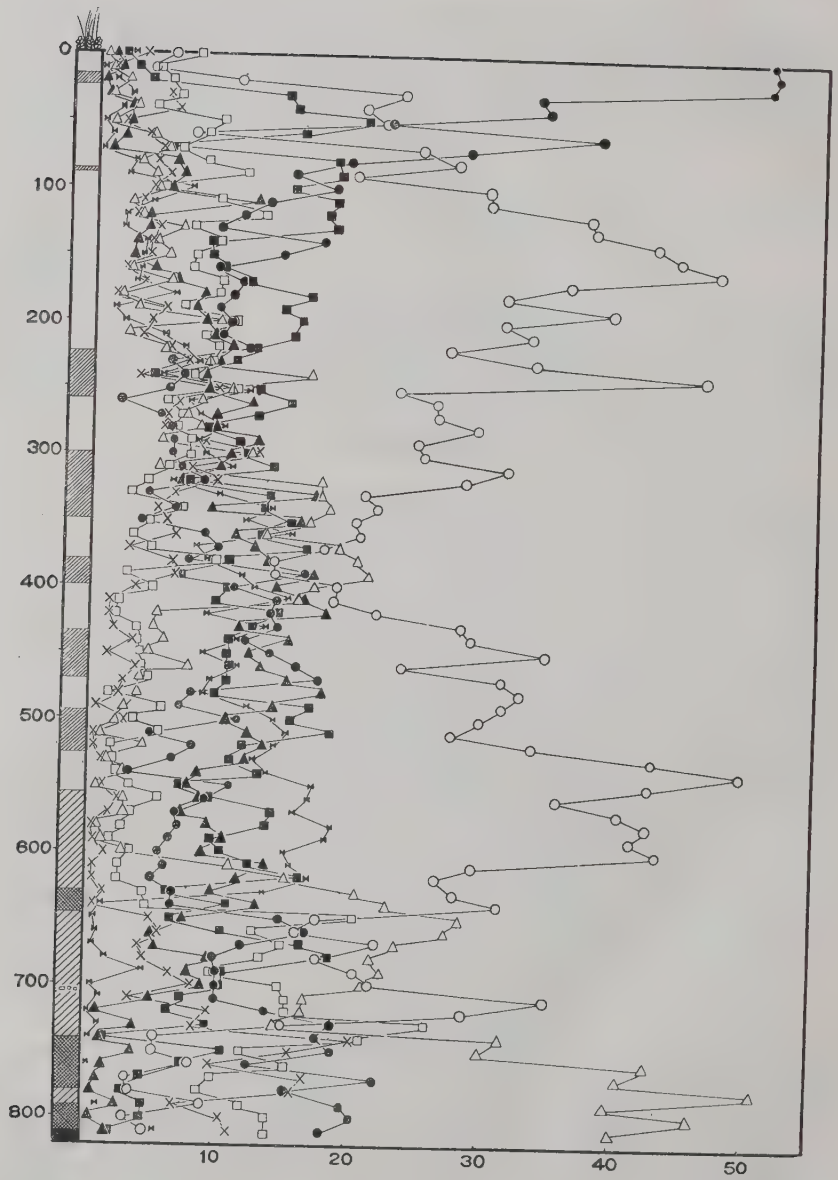
The percentage of *Tsuga* is generally low. It is below 10% in several layers near the surface, falls to about 5% in middle layers and rises a little downward from 650 cm reaching 26% at 730 cm depth.

Picea has low percentages in upper layers, but the percentage abruptly rises between 320 and 410 cm. From 420 to 600 cm it is low and from 610 cm it again rises abruptly, reaching 53% at 780 cm.

Fagus shows rather high percentages (above 15%) between 300 and 500 cm, but in other layers the percentage is generally low.

Betula like *Fagus* generally has low percentages, but at 100, 400 and 600 cm respectively shows rather high percentages (above 15%). *Ulmus* has percentages above 10% from 400 to 630 cm, but in other layers the percentage is low.

Quercus shows higher percentages throughout the whole layers, compared with those of other pollens. It is 5.5% in the uppermost layer, but increases


0
100
200
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50

with the depth, becomes more than 20% between 100 and 360 cm, attains 47.6% at 160 cm. It decreases to 14% at 380 cm depth, but again rises sharply at 400 cm, reaching as high as 49% at 540 cm. But, beyond this layer, the rate falls gradually, reaching as low as below 10% near the bottom.

From these results following division of the periods was proposed.

Pinus period	0～100 cm
Upper *Quercus* period	100～310 cm
Upper *Picea* period	310～410 cm
Lower *Quercus* period	410～640 cm
Lower *Picea* period	640～805 cm

b) Oomine-numa Bog

Near the summit of Mt. Oomine, which rises in Tone-gun, Gunma prefecture, 4 km west of Kamimoku Station of the Jōetsu line, there is a small lake, named Oomine-numa (1100 m above the sea level), where a bog is formed. This bog has a peat layer of 860 cm in depth, from which the writer obtained pollen samples of various species. By the analysis of the pollens of major species such as *Pinus, Picea, Abies, Tsuga, Quercus, Fagus, Corylus, Betula, Ulmus, Juglans, Pterocarya* and several species of Ericaceae, a pollen diagram was constructed (Fig. 3).

Pinus shows high percentages of over 70% from the surface to the 50 cm depth, and especially in the 20 cm layer it attains 87%. It is 20～40% between 60 and 140 cm, and decreases with the depth, not surpassing 10% in the layers deeper than 200 cm.

The percentages of *Picea* are as low as below 5% from the surface to the 300 cm layer, and then rise above 10% ; but in layers deeper than 420 cm they again decrease to as low as below 5% and reach the lowest.

Abies shows low percentages as deep as 150 cm, but in deeper layers it shows often more than 10%, from 300 to 400 cm above 15%, and at the 340 cm depth attains 70%. In layers deeper than 400 cm it shows low percentages and reaches the lowest.

The percentage of *Tsuga* is below 10% from 60 to 80 cm, and in other layers it is almost below 5%.

Betula shows the percentages of above 10% in layers of 540～640 and 780～860 cm, but in the other layers as low as below 10%.

The percentage of *Quercus* attains 27% in the 200 cm layer, but in the

Fig. 2. Pollen diagram from Yashimaga-hara bog. The pollen frequencies are marked off on abscissa, the depths under the surface are indicated on ordinate. Pollen symbols are shown as follows: –●– *Pinus*; –×– *Abies*; –△– *Picea*; –■– *Betula*; –□– *Tsuga*; –▲– *Fagus*; –○– *Quercus*; –◆– *Alnus*; –⋈– *Ulmus*. The percentages of *Corylus, Juglans* and *Pterocarya* are omitted from this diagram. The column parralleled with the diagram is the peat layers represented as follows: ⌈ ⌉ peat layers of the part of high bog: |////// weathered layers: |//// peat layers of the part of low bog; ▒ highly decomposed peat layers; ▬ sand layer.

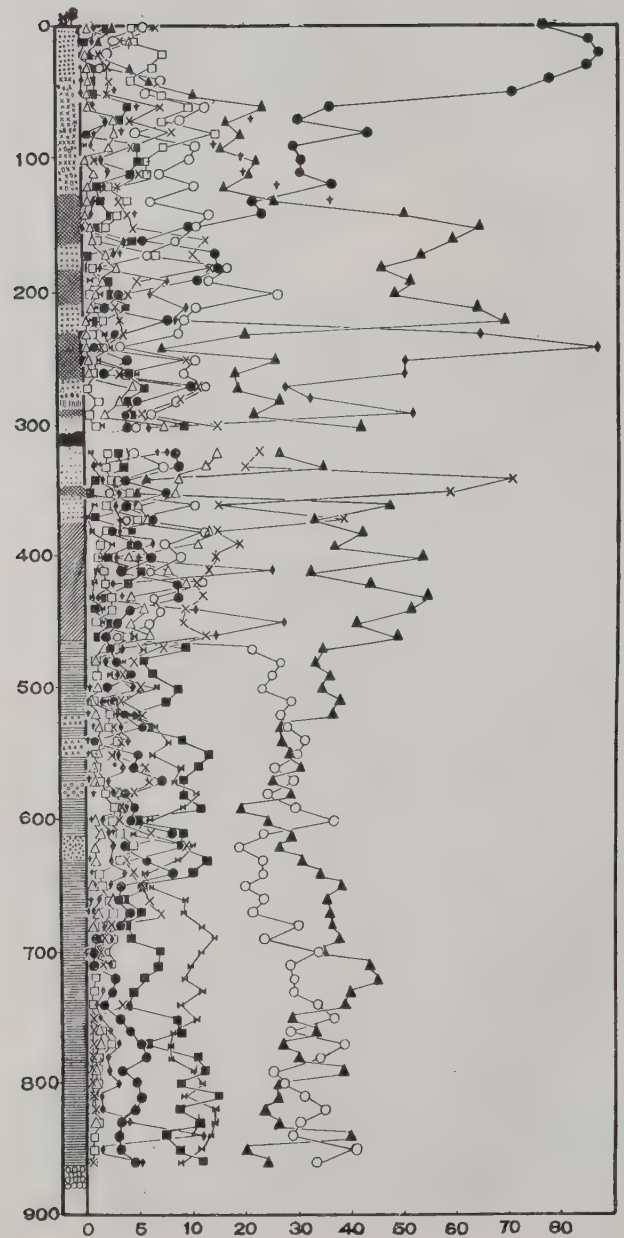

Fig. 3. Pollen diagram from Oominenuma. Ordinate: depth of peat layer (cm), Abscissa: pollen percentage. Pollen symbols are the same with those in Fig. 2. The column paralleled with the diagram is the peat layers represented as follows: ×××× ×××× *Carex* peat; //// *Sphagnum-Carex* peat; Compact *Sphagnum-Carex* peat; highly decomposed peat in which macro-relics can not be found; Sand layer.

upper half it is generally low, and in the lower half it becomes above 20%.

Fagus shows percentages of about 20% from 60 to 120 cm layers, but in the 220 cm layer it attains 69%. From 230 cm it decreases a little; but in the layer from 360 to 520 cm it becomes above 30%, and in 400, 430 and 440 cm layers attains the high percentages of above 50%. Also in the lower half, *Fagus* always shows the percentage of above 20%, becoming higher than *Quercus* at 610 and 740 cm.

The percentage of *Alnus* rises abruptly between 230 and 290 cm, attaining as high as 86% at 240 cm, and also showing peaks of 27% at 450 cm and 25% at 410 cm, but it is generally below 10%.

Ulmus shows low percentages of below 5% in the layers above 500 cm depth, and in the layers below 500 cm it is lower than 10%.

From the above mentioned results, the writer divided the whole period as follows:

Pinus period	0～120 cm
Upper *Fagus* period	120～220 cm
Alnus period	220～290 cm
Abies period	290～370 cm
Lower *Fagus* period	370～520 cm
Fagus-Quercus period	520～650 cm
Fagus-Ulmus period	650～860 cm

B) The Rate of Peat Growth

Peat layers in Northern Europe and North America are generally formed on glacial deposits which are chronologically known. Consequently, the number of years required for the deposition of peat can be estimated. But in Japan, where no conspicuous glacial deposits are found, such a method of determination is inapplicable. Therefore the writer determined the ages of peat layers by the use of volcanic ash layers inserted in peat deposits. The date of the formation of volcanic ash layers can be known from the records in the literature. In the bogs situated far from any volcano the content of ash is naturally low, so the ash contained in peat layers was detected microscopically, and from the number of years which have elapsed from the eruption till now and the thickness of the peat layer the rate of peat growth was calculated.

By these means the writer obtained the rate of peat growth of Yoshiga-daira, Asama-yama, Kakuman-buchi in Akagi, Yahazu-daira, Usagi-shima in Nikko, Kinu-numa and Kobuga-daira bogs.

a. Yoshiga-daira Bog

Yoshiga-daira bog (1500 m above the sea level) is situated near the summit of Mt. Kusatsu-Shirane. The plant communities on this bog were often destroyed by the eruption of Mt. Shirane. The present plant communities were formed on the volcanic ash layer produced by the eruption in 1881 and consist chiefly of *Eriophorum*-sociation. *Sphagnum*-sociation can also be seen scattered

among them. The writer measured the growth of the peat layer and obtained the following results (Table 1).

Table 1. The thickness of the peat layers formed during the time from 1881 to 1948 at Yoshiga-daira.

	Station number	Thickness of peat layer, mm	Average annual growth, mm
Eriophorum-sociation	1	67	1.00
	2	71	1.06
Sphagnum-sociation	1	71	1.06
	2	69	1.03
	3	63	0.94
	4	67	1.00
	5	65	0.97
	6	62	0.93
Average		66.9	0.99

b. Asama-yama Bog

Small bogs are found scattered at Juni-no-mori about 7 km north of the summit of Mt. Asama.

The writer estimated the total number of pollens contained in a given quantity of the peat of each layer (Fig. 4).

It can be seen from this that in the peat layers lying between the surface and at the depth of about 18 cm but a small amount of pollens were found, whereas further below the quantity of pollens increased abruptly, forming a conspicuous borderline at about 18 cm depth. Such features might suggest that an extensive destruction of plant communities around the bog might have taken place at the time of the formation of peat at 18 cm depth. It appears probable that for the paucity of pollens in upper layers the eruption of Mt. Asama in 1783 might be responsible, when a great quantity of lava flew out from the crater northward to the bog, forming a great lava-stream locally called "Oni-oshidashi". A northward flow of lava in such a large scale seemed not to occur either before or after 1783 (Yagi 1921, 1930, 1935).

According to the records on the eruption of Mt. Asama from 1783 to 1935 (Yagi 1921, 1930, 1935), especially violent eruptions occurred 6 times; namely in 1783, 1803, 1815, 1869, 1894 and 1929. According to the same records the prevailing west wind limited the falling of the ash mostly to the eastern and south-eastern areas, and in a small eruption the ash did not fall ordinarily on the bog which is situated to the north of the mountain.

A comparison of the ash layers with the records of the ash fall shows that the ash of 1783 corresponds to A_1, 1803 to A_2, 1815 to A_3, 1869 to A_4, 1894 to A_5 and 1929 to A_6 respectively (Fig. 4).

The results of the measurement of the thickness of peat layers above A_1 are as Table 2.

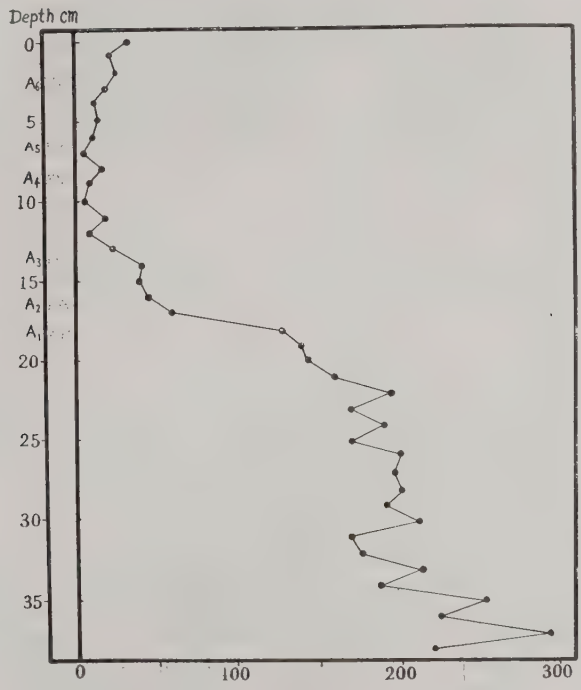

Fig. 4. Pollens contained in the peat layers of Asama-yama bog. Ordinate: depth of peat layer (cm), Abscissa: total number of pollens per 1 g. in peat layers. The dotted parts (A_1–A_6) are the layers containing volcanic ash.

The thickness of the peat layers interposed between volcanic ash layers are as Table 3.

c. Kakumanbuchi Bog in Akagi

This is a bog near Lake, Oonuma, on Mt. Akagi, consisting of *Sphagnum* peat layers. It is situated 60 km east of Mt. Asama, and it has been known that the ash of this volcano often reached this area.

From several layers of the *Sphagnum* peat, in which seemingly no volcanic ashes were contained, a minute amount of them, probably of idiomorphic feldspar, could be detected under a microscope. From the nature of these volcanic ashes and the direction of the wind at the time of the eruption of Mt. Asama, as given in ancient records (Yagi 1921, 1930, 1935), it appears not absurd to consider that the volcanic ashes found in the peat of this bog might have their origin in the eruption of Mt. Asama. Based on this consideration the rate of peat growth at Akagi-Kakumanbuchi bog was assessed as follows (Table 4).

Table 2. The thickness of peat layers of Asama-yama bog formed since the fall of volcanic ash in 1783 up to 1949.

On *Carex-Eriophorum*-sociation			On *Sphagnum*-sociation		
Station number	Thickness of peat, mm	Amount of yearly growth, mm	Station number	Thickness of peat, mm	Amount of yearly growth, mm
1	182	1.09	1	168	1.01
2	180	1.07	2	174	1.05
3	177	1.06	3	165	0.99
4	185	1.11	4	170	1.02
5	190	1.14	5	162	0.98
6	188	1.13	6	160	0.96
7	175	1.05	7	166	1.00
8	182	1.10	8	160	0.96
			9	162	0.98
			10	160	0.96
Average	182.4	1.09	Average	162	0.99

Table 3. The peat layers of Asama-yama bog measured at two stations: *Carex-Eriophorum*-soc. and *Sphagnum*-soc.

Peat layers interposed between volcanic ash layers*	Age of peat layer, year**	*Carex-Eriophorum*-soc.		*Sphagnum*-soc.	
		Thickness of peat layer, mm	Average yearly growth, mm	Thickness of peat layer, mm	Average yearly growth, mm
A_6.....A_5	35	31	0.89	32	0.91
A_5.....A_4	25	18	0.72	20	0.80
A_4.....A_3	54	51	0.94	48	0.89
A_3.....A_2	12	13	1.08	14	1.17
A_2.....A_1	20	18	0.90	18	0.90

* $A_1 \sim A_6$: volcanic ash layers as shown in Fig. 4.
** Time interval in year between successive ash depositions.

Table 4. The rate of peat growth at Kakumanbuchi bog.

Time of eruption	Years elapsed since the eruption up to 1949	Thickness of peat layer, mm	Average yearly growth of peat, mm	Remarks
1894	55	60	1.09	
1869	80	80	1.00	*Carex-Sphagnum*-soc.
1803	146	145	0.99	
1783	166	165	0.99	

Table 5. The rate of peat growth at Yahazu-daira bog.

*Time of eruption	Years elapsed since the eruption up to 1949*	Thickness of peat layer, mm	Annual growth of peat, mm	Remarks
1932	17	15	0.88	
1926	23	20	0.86	*Sphagnum*-soc.
1902	47	40	0.85	
1882	67	65	0.97	
1783	166	170	1.02	Volcanic ash from Mt. Asama-yama

* 'The chronological table of science' published by the Central Meteorological Observatory of Japan (1943).

d. Yahazu-daira

Yahazu-daira bog is a small bog, 4 km south of Mt. Kusatsu-shirane, and 1600 m above the sea level. It is in the course of transition from marsh to low bog, and most of its surface is covered by floating islands.

The voloanic ash in the peat layer of this bog is considered as due to the eruption of Mt. Kusatsu-Shirane. Eruptions were known to have occurred four times in all, namely in 1882–1900, 1902–1905, 1926 and 1932. Volcanic ashes were examined as above and the results are presented in Table 5.

This bog is situated about 25 km north of Mt. Asama. As already stated, however, the ash from the volcano is usually blown east or south by the westerly wind and does not reach this bog which lies northward to the volcano. But in the eruption of 1783 ash was recorded to have been blown northward, so the lowest layer may be inferred to have been formed by the ash of Mt. Asama.

e. Nikko-Usagishima, Kinu-numa and Kobuga-daira Bogs

These three bogs are all within reach of the volcanic ash from Mt. Nikko-Shirane. Usagi-shima bog is a small bog at the lake side of Yunoko, 4 km east of Mt. Shirane. Kinu-numa bog lies 10 km north of the mountain, 2000 m above the sea level and represents a rather typical form of a high bog. Kobuga-daira bog is a small bog situated 20 km east of the mountain, 6 km east of Ashio-machi. In these bogs, however, five layers of volcanic ash from their surface are found. Of these layers the first and the second one (U_1, U_2, Ki_1, Ki_2, Ko_1, Ko_2) from above as well as the fourth and the fifth one (U_4, U_5, Ki_4, Ki_5, Ko_4, Ko_5,) are found respectively lying close to each other. These four layers of ash are considered to have their origin in the volcanic eruptions of Mt. Shirane in 1889, 1872–73, 1649 and 1625 respectively (Table 6). The third layer (U_3, Ki_3, Ko_3,) lying at the depth of 15–16 cm is separated from the others. The volcanic eruption corresponding to this ash layer could not be found in the records of the eruption of Mt. Shirane. As mentioned above, however, an ash layer is found in Asama-yama bog (Table 2) and Yahazu-daira bog (Table 5) respective-

Table 6. The rate of peat growth at Nikko-Usagishima, Kinu-numa and Kobuga-daira bogs.

	Volcanic ash layer	Time of eruption*	Years elapsed since the eruption up to 1948	Thickness of of peat layer, mm	Annual growth of peat, mm
Nikko-Usagishima bog	U_1	1889	60	65	1.08
	U_2	1872–73	77–78	80	1.04
	U_3	1783	166	155	0.93
	U_4	1649	300	300	1.00
	U_5	1625	324	335	1.03
Kinu-numa bog	Ki_1	1889	60	65	1.08
	Ki_2	1872–73	77–78	90	1.15
	Ki_3	1783	166	155	0.93
	Ki_4	1649	300	310	1.03
	Ki_5	1625	324	355	1.10
Kobuga-daira bog	Ko_1	1889	60	60	1.00
	Ko_2	1872–73	77–78	95	1.22
	Ko_3	1783	166	155	0.93
	Ko_4	1649	300	295	0.98
	Ko_5	1625	324	335	1.03

* 'The chronological table of science' published by the Central Meteorological Observatory of Japan (1943).

ly which lies equally at the depth of 15–16 cm and may be regarded as due to the eruption of Mt. Asama in 1783. The volcanic ashes from Mt. Asama are usually blown away either southward or eastward, but in the case of the great eruption of 1783, it is recorded that the ashes were also blown northward. As to the other volcanoes situated not very far from these three bogs, such as Mt. Bandai and Mt. Zao, no eruptions are recorded at that time which were likely to supply ashes to the bogs. In view of these considerations the writer is of the opinion that the volcanic ash layer (U_3, Ki_3, Ko_3) might have its origin in the great eruption of Mt. Asama in 1783.

The rate of peat growth was estimated as above mentioned by virtue of volcanic ashes and is shown in Table 6.

f. Rate of Peat Growth

From the volcanic ash layers interposed in the peat layers and the dates of eruptions of the volcanoes which are considered as responsible for these ashes in Yoshiga-daira, Asama-yama, Kakumanbuchi in Akagi, Yahazu-daira, Kinu-numa, Usagishima in Nikko and Kobuga-daira bogs, the writer measured the rate of the growth per year of the peat layer. From such estimations the average value of 1.01 mm/year was calculated. As far as the present investigations go,

it appears probable that the rate of peat growth in the bogs of the central hill districts of Japan, lying about 1000–1500 m above the sea level, may be about 1 mm/year, the rate being applicable to both Yashimaga-hara and Oomine-numa bogs which are situated on the same level.

5. Discussion

A) Characteristics of Yashimaga-hara and Oomine-numa Bogs

As stated above Yashimaga-hara bog possesses peat layers 8.05 m deep, consisting chiefly of *Sphagnum* peat and not containing volcanic ashes. It appears that the plant communities of this bog were not destroyed by volcanic activities and maintained uninterrupted since the beginning of the bog.

Oomine-numa bog presents nealy the same aspects as Yshimaga-hara bog except that it has peat layers 8.6 m deep. The elevation of Yashimaga-hara bog is 1500 m and that of Oomine-numa bog is 1100 m above the sea level. Both are situated on the lower part of the coniferous forest zone and simultaneously on the upper part of the deciduous forest zone.

In view of the fact that these two bogs have well developed peat layers of more than 8 m in depth and that their environmental conditions are in the main similar, it seemed appropriate from the date obtained from these bogs to make some considerations on the forest succession.

B) Forest Succession

In Japan a number of studies on pollen analysis have hitherto been made, such as: Jimbo (1936) on the humus (20 cm deep) and peat (35 cm deep) in Hakkoda; Nakamura (1942, 1948, 1949) on Akami-zawa bog (180 cm deep), Senninta bog (20 cm deep), Yachi (70 cm deep) in the Botanical garden in Hakkoda, Ozega-hara (60 cm deep), and Tosa (210 cm); Miyai (1933, 1935, 1938) on Kirishima-yama bog (90 cm deep), Usagi-shima in in Nikko (100 cm deep), Yakushima (60 cm deep); Yamazaki (1937, 1942, 1943) on bogs in Saghalien and Hokkaido; Numata and Tamada (1936, 1939) at Yagumo-daira (130 cm deep), Uchimi-yama (90 cm deep), Midoroga-ike (25 cm), Hacho-daira (210 cm) bogs near Kyoto and bogs in Mt. Ontake and Mt. Tateshina. These studies, except those of Yamazaki, and Numata and Tamada, were made on bogs with very thin peat layers, and therefore, their results can not be compared directly with those of the writer. Yamazaki's studies, made in Saghalien and Hokkaido, can hardly serve my purpose as his studies were done in the far north where climatic conditions are different from those of the cenral part of Japan.

Numata and Tamada made pollen analyses with the peat layers of 3.7 m in thickness of Yashimaga-hara, and from the results obtained they concluded that forest successions may be as follows: from the past to the present; *Fagus-Quercus* period- - - -*Carpinus-Picea* period- - - the 2nd *Quercus* period- - - - *Pinus* period. Further, as a result of pollen analyses with peat layers of 3.6 m in thickness of Onna-ike in Kyoto, they postulated the existence of *Pinus-*

Picea-Abies-Tsuga period preceding the above mentioned *Fagus-Quercus* period. When the results of Numata and Tamada are compared with those obtained by the writer at Yashimaga-hara bog it becomes probable that the period ascertained by them at Yashimaga-hara bog might be more recent than the writer's Upper *Picea* period and that the period by them at Onna-ike might correspond to the writer's Upper *Picea* period at Yashimaga-hara bog.

As a result of pollen analyses of Yashimaga-hara bog the writer considers the forest succession as follows:

Lower *Picea* period---Lower *Quercus* period---Upper *Picea* period---Upper *Quercus* period---*Pinus* period.

In peat layers of 8.06 m in thickness of Yashimaga-hara bog neither sand nor volcanic ash layer was found, but they consisted chiefly of *Sphagnum*. Hence it appears likely that the formation of peat proceeded uninterruptedly. Consequently the above mentioned formula of forest succession at Yashimaga-hara bog might be taken as the standard for regions about 1500 m above the sea level in the central part of Japan.

By the pollen analyses of Oomine-numa bog, the writer proposed the forest succession as follows:

Fagus-Ulmus period---*Fagus-Quercus* period---Lower *Fagus* period---*Abies* period---*Alnus* period---Upper *Fagus* period---*Pinus* period.

Oomine-numa bog lies at about 1100 m above the sea level, much lower than Yashimaga-hara bog, and consequently more other species were found which did not exist in the latter bog. Therefore, the formula of the forest succession became somewhat complicated, but it can be regarded as a modified form of the Yashimaga-hara formula. Namely, the lower *Picea* period and upper *Picea* period of Yashimaga-hara correspond to *Quercus-Ulmus* period and to *Abies*, *Alnus* period of Oomine-numa respectively.

The comparison of these results are shown in Fig. 5.

C) Forest Succession and Climatic Change

Numerous researches concerning the vertical distribution of coniferous and deciduous trees in Japan have been made by many workers as follows: Koizumi (1913) in Mt. Ontake, Takenaka (1931) in Mt. Shirouma, Takeda (1931) in Mt. Fuji, Arikawa (1935) in Mt. Zao, Nakai and Ito (1936) in Nikko, Nakano (1942 b, c) in Mt. Yatsugatake, Honda and Tobita (1941) in Yashimaga-hara, Horikawa (1930) and Yoshioka (1937, 1938, 1943) in Mt. Hakkoda, etc.

From these data the *Fagus crenata*-soc. is found from 800 m to 1500 m above the sea level in the central part of Japan. The *Quercus crispula*-soc. extends below the *Fagus crenata*-soc. and the maximum height of its distribution lies at about 1000 m above the sea level. The lower limit of distribution of *Abies Veitchii*, *Abies Mariesii*, *Tsuga Sieboldii*, and *Picea jezoensis* is 1500 m above the sea level.

1) Yashimaga-hara Bog

Yashimaga-hara bog lies about 1500 m above the sea level and is situated below the coniferous forest zone and above the *Fagus* zone. It is common in the central part of Japan that the *Fagus crenata*-soc. contains *Quercus crispula*, whose lower limit is far below that of *Fagus crenata* (Nakano 1942 b). At present, *Quercus crispula* cannot be found around Yashimaga-hara bog. But the pollen analytical studies of the writer indicated the presence of upper *Quercus* and lower *Quercus* periods. These facts may be taken to show that *Quercus crispula* had the wide distribution around the bog in the past, and that it had grown in higher places than at present. Consequently it may be said that it was warmer in those ages.

At present *Picea jezoensis* is found in the forest on Kuruma-yama (1925 m), but not around the bog. From this fact and the present distribution of *Picea* in Japan, this plant was supposed to have grown in the *Picea* period of the writer in lower places than is found now, and contrary to the *Quercus* period, the *Picea* period was considered as colder than at the present.

In the studies of the writer at Yashimaga-hara and Oomine-numa bogs, it was found that near the uppermost layers of both bogs, a large amount of pollens of *Pinus densiflora* was found and that its frequency of occurrence exceeded 70%. The increase in amount of the pollen of *Pinus densiflora* near the surface was noted by the writer also at Odoriba (1940), Sugadaira (1949 a), Nozori-ike and Kakumanbuchi bogs. The same features were observed by Numata

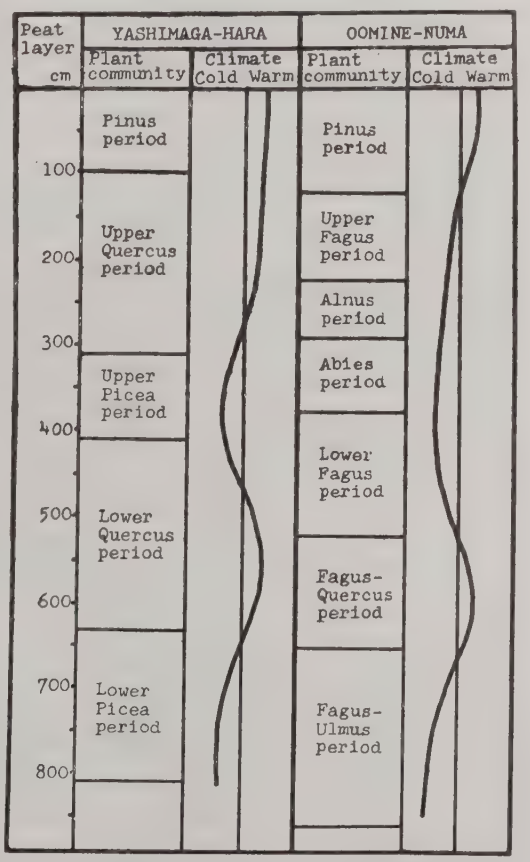

Fig. 5. Forest succession of Yashimaga-hara and Oomine-numa bogs and climatic changes underlying it.

and Tamada at Yashimaga-hara and Onna-ike bogs (1939) and also by Miyai at Nikko-usagishima (1933) and Kirishima-yama (1938) bogs.

Yoshii and Yoshioka (1940) found *Pinus densiflora* growing on Maedake (500 m above the sea level) of Mt. Hakkoda. According to these authors such a distribution may be regarded rather as an exceptional one brought about by the peculiar environmental conditions. Yoshii (1939) observed on Mt. Bandai the existence of *Pinus densiflora* up to the height of 1200 m, and again considered that it might be due to an unusual environmental condition, such as naked ground formed volcanic activities. Yoshii maintained the view that the proper habitat of *Pinus densiflora* mitht be rather low and could only be distributed to such height under peculiar conditions mentioned above.

On the other hand Nakano (1942 b) observed that *Pinus densiflora* grew abundantly at the foot of Yatsugatake about 1300 m above the sea level forming a *Betula Taushii-Pinus densiflora* sociation and that even invaded the *Fagus* zone lying higher than the above mentioned sociation. Nearly the same features were noticed by the writer on Kiriga-mine near Yashimaga-hara and Oomine-numa, and also in several other places of the central part of Japan.

According to Masamune (1936), *Pinus densiflora* is distributed in the area between the northern extremity of Honshū and Yakushima island and is rather regarded as a plant of the warm territory.

Consequently, judging from the observations of Nakano (1942 b) and of the writer that *Pinus densiflora* grows at present in fairly higher places of the central part of Japan, and also from those of Masamune (1936) on the horizontal distribution of this plant, it may be considered likely that the climate in the *Pinus* period of the writer as represented by *Pinus densiflora* might have been rather warm.

From these considerations the forest succession and the climatic change of Yashimaga-hara and Oomine-numa are illustrated as follows (Fig. 5).

2) Oomine-numa Bog

Oomine-numa bog is situated 1100 m above the sea level, and on the lower part of the *Fagus* zone. From this, it may be considered probable that in the upper and lower *Fagus* periods as evidenced in this bog, the climate was a little colder than it is at present.

Beyond the depth of 500 cm of the peat layer, the percentage of *Ulmus* pollen increases. In Oomine-numa bog and its vicinities two species of the genus *Ulmus*, namely, *U. Davidiana* and *U. laciniata*, are found at present. Hence it appears likely that the pollens of *Ulmus* contained in the peat layer might be derived from these species. According to Nakai and Ito (1936), these species are found in the *Fagus* zone in Odashiro-hara and Mt. Nantai in Nikko. It is also known that at the lake side of Yunoko and its neighbourhood which are the upper limit of *Fagus* zone, big trees of *Ulmus Davidiana* are found among many subalpine plants as *Tsuga sieboldi*. According to Nakai (1928) *Ulmns Davidiana* and *Ulmus laciniata* grow among deciduous trees in Kamikoochi (1500 m) in Nagano prefecture and are also very common in Ho-

kkaido and Saghalien. The present writer also observed on the hill districts bordering Gunma and Niigata prefecturs and on Oze, *Ulmus laciniata* growing in the upper part of *Fagus* zone and often even beyond this zone.

From these facts the writer holds an opinion that in Oomine-numa bog the climate in the *Fagus-Ulmus* period as shown by pollen analyses might have been colder than in the *Fagus* period.

Based on the findings that the coniferous zone descended in the *Abies* period to the level of the present *Fagus* zone, the climate in the *Abies* period might have been colder than at present.

The representative species of the *Alnus* period seems to be *Alnus hirsuta* var. *sibirica* as it grows now abundantly on Mt. Oomine. This species also grows at present in higher places than Mt. Oomine, such as the upper part of the *Fagus* zone over 1200 m high on the border zone of Gunma and Niigata prefectures. Consequently it may be considered probable that the climate in the *Alnus* period of the writer might have been somewhat colder than it is at the present.

The climate of the *Pinus* period of this bog is considered to have been slightly warmer than the upper *Fagus* period. As the discussion on the climate of this period has already been made in a chapter on Yashimaga-hara bog, it will not be repreated here.

The pollen of *Betula* was found in the peat of this bog in an appreciable amount. Major species of the genus *Betula* seems to be *Betula Ermani*. According to Yoshioka (1943), this plant is distributed from 700 m to 1500 m above the sea level in Mt. Hakkoda. According to Arikawa (1935) this plant begins to aprear along the upper limit of *Fagus crenata-Quercus crispula* sociation, that is, from 1100 m to 1200 m, and exhibits its luxuriant growth between 1200 m and 1400 m above the sea level. Nakano (1942 b) stated that the optimum habitat for *Betula Ermanii* lies between 1600 m and 2400 m above the sea level. *Betula Ermanii* has been considered by many researchers as a sun plant. It does not grow in a dense forest but prefers an open and sunny ground made by a fire avalanche. Because of such a wide range in vertical distribution and the peculiar way of habitation, *Betula Ermanii* does not seem suitable as a standard for the determination of the climatic changes.

As can be seem from Fig. 5 the climatic changes approached from the study of pollen analysis of Yashimaga-hara and Oomine-numa bogs are similar to each other. As Oomine-numa bog is situated lower than Yashimaga-hara bog, the *Abies* period appeared in Oomine-numa bog in place of the *Picea* period of Yashimaga-hara bog. Besides there existed periods represented by *Alnus* and *Ulmus* in the former. Thus the climatic change and the succession of plant community in Oomine-numa bog appear rather complicated as compared with those of the Yashimaga-hara.

D) The Rate of Peat Growth

On the rate of peat growth, numerous researches have hitherto been made and exceedingly manifold values of the rate of peat growth have been obtained,

ranging from 2 to 1665 yers required for the deposition of one foot of peat (Sears and Janson 1933).

Apart from the extremes, there is a tendency among the estimates to cluster about values of from 100 to 400 years per foot for average well consolidated peat: Lesquereux (1885) suggested the growth rates of from 300 to 400 years per foot for peats of various regions. Dacknowski (1912) obtained estimates of 200 years per foot of peat formation as a result of extensive researches on peat deposition in the central states of America.

In Europe the postglacial climates have been accurately dated by Geer's measurement of clay layers. Since such a method of measurement had not been generally available in America, Sears and Janson (1933) made use of the following special method. In Bucyrus bog (northern Ohio), thin layers consisting of algal jelly and sedge leaves were found in alternating position near the surface of the peat layer; 25 pairs of the lamination were present in 1 inch of thickness. Assuming that each pair was formed yearly in spring and autumn, it was estimated that a period of at least 300 years might have been required for the deposition of 1 foot of the peat layer.

To secure further date, isolated spruce and larch trees living in the bog were selected. The age of each was then determined by means of an increment borer which can remove a small core wood from circumference to center without injuring the tree. Pits were sunk below each tree and a careful search made for needles as far down as they could be found. The needles in the deepest of the pear layers may be the oldest leaves which were fallen from the tree in the beginning of its life. From the thickness of peat layer between the needles and the present surface, and the number of years which was obtained by an increment borer, the rate of peat growth was calculated.

Summarizing these measurements, they concluded that the mean rate of peat growth of 1 to 1.5 mm per year would be probable during the period of the past several years.

In Japan Miyai (1933), in his researches on the peat layer of Usagi-shima bog in Nikko, stated

Table 7. Specific gravities of dried peat from different layers of Yashimaga-hara bog. Layers from the surface to the 550 cm belong to high bog, among which there are 6 weathered layers marked by +, while △ shows layers of low bog; macro-relics could not be detected. * layer with sand.

Depth cm	Specific gravity	Remarks
0	0.11	
50	0.14	
100	0.28	+
150	0.12	
200	0.11	
250	0.43	+
300	0.32	+
350	0.35	+
400	0.27	
450	0.40	+
500	0.36	+
550	0.26	
600	0.34	△
650	0.57	△
700	0.51	△
750	0.78	△ *
800	1.20	△ *

that the volcanic ash layer which was found at the depth of 20 cm below the surface had been formed by the eruption of Mt. Nikko-shirane in 1872～73, and that if this be correct the 20 cm of peat would have grown for 60 years, namely the rate of growth would be 0.3 cm per year. Further, on the assumption that the volcanic ash layer at 50 cm depth below the surface was due to the eruption of 1647, the peat growth would be 30 cm for 230 years: namely 0.1 cm per year.

As was shown in chapter (B), the writer determined the rate of peat growth of volcanic ash layers contained in peat layers. Several such determinations yielded the average growth rate of peat of 1 mm per year. This value agrees approximately with the results of Miyai (1933), Sears and Janson (1933).

The degree of compression of the peat increases naturally with the depth. This fact must be taken into consideration. As a measure of compression degree the writer measured the specific gravity of each of the peat layers and obtained the following results (Table 7).

The ratio of the specific gravity of peat at 50 cm depth to that of 550 cm depth (this being the bottom of the part belonging to the high bog) was found 1:1.8.* The specific gravities of the peat of the low bog, lying lower than 600 cm below the surface, are 2,5～4.1 times larger than that of the peat at 50 cm depth and the value increases further with the depth (750～800 cm).

That the specific gravities of these peat layers are 2.5～4.1 times as large as those of the upper peat layers is due to the fact that the formers were formed during the course of the transition of a shallow marsh into land and consequently a large quantity of humus and muddy substances were carried down into marsh from the adjoining parts, thus contributing to the formation of the peat. Because of the large specific gravities of these peat layers, it would appear that the time required for the formation

Table 8. Specific gravities of dried peat from different layers of Ozega-hara by Yamagata (1956).

Depth cm	Specific gravity	Remarks
25	0.21	
50	0.56	These contain pumice and volcanic ashes.
75	0.78	
100	0.46	
125	0.45	
150	0.32	
175	0.25	
200	0.27	
225	0.51	These contain volcanic ashes.
250	1.32	
275	0.42	
300	0.25	
325	0.28	
350	0.36	
375	0.41	
400	9.62	Weathered layer.
425	0.49	
450	0.45	
475	0.86	With sand.

* Weathered layer—the layer where decomposition of peat proceeded more vigorously than the neighbouring layer; it is omitted from the comparison of specific gravity as exception.

of these peat layers was in proportion also 2.5~4.1 times as long. But in reality, this could not be the case, for, as confirmed by the writer the major part of these peat layers is composed of the remains of Cyperaceae, of which the yearly production is larger than that of *Sphagnum*. Therefore, the time required for the formation of these peat layers cannot be so long as is suggested from the values of their specific gravities of 2.5~4.1. The time required for the formation of these lower layers would be only twice as long as that of the upper layers, namely nearly 2 years per mm of the lower peat layers as against 1 year per mm of the upper peat layers.

Yamagata (1956) measured the specific gravities of peat in various layers of Ozega-hara bog and obtained the following results (Table 8).

According to the preceding Table 8, the minimum value of specific gravity got at 25 cm depth is 0.21 and the maximum at 425 cm depth is 0.49. The ratio between them is 1:2.3. The layers at 50–75 cm and 225–275 cm depths contain pumice and volcanic ash respectively, and the layer at 400 cm depth is a weathered one: because of their exceptionally high values of specific gravity resulting from the above mentioned condition these layers were left out of consideration. This ratio approximates that one got by the writer at Yashimaga-hara bog between its upper and lower peat layers. From these results by Yamagata concerning the specific gravity of peat just as well as from those by the writer at Yashimaga-hara bog, it is ascertained that the rate of compression of peat expressed as the ratio between the specific gravities of the upper and lower layers is not so high as is supposed but is only from two to three.

From these considerations it becomes likely that for the formation of the lower peat layers of 2 m in thickness of Yashimaga-hara bog about 4000 years were required and for the higher peat layers of 6 m in thickness of the same bog, 6000 years; thus, about 10,000 years needed for the formation of the whole bog.

The peat layer in Oomine-numa bog consisted of nearly the same plants as those of Yashimaga-hara bog and appeared to have passed through the same course of development and the time required for its formation is considered approximately the same as that for Yashimaga-hara bog.

E) Comparison with Climatic Changes in Northern Europe with the Results of the Writer

As a results of the extensive researches on the peat layers, Blytt (1876) considered the difference of peat quality as due to the difference of climate and he put forward a theory of the climatic changes in Northern Europe. Later Sernander (1910) modified this theory slightly and established a standard chronological scale, now kown as Blytt-Sernander system, as follow:

1.	Pre-boreal	(~6800 B.C.)	Cool-humid.
2.	Boreal	(6800~5000 B.C.)	Warm-dry, continental.
3.	Atlantic	(5000~3000 B.C.)	Warm-humid climatic optimum.
4.	Subboreal	(3000~850 B.C.)	Drier, continental.
5.	Subatlantic	(850 B.C.)	Humid, less warm.

Köppen (1903) who compared Millankovitch's solar curve with Blytt-Sernander's scale, stated that the climatic changes as inferred from Milankovitch's date agreed fairly well with that of Blytt-Sernander.

Von Post (1930) proposed on the basis of his pollen analytical data a three-phase temperature change in postglacial period as follows:

1. The period of increasing warmth: the stage of the approach of the warm period, characterized by the appearance and the primary increase of the relatively heat-requiring trees of different kinds.

2. The period of maximum warmth: the stage of the culmination of these forest elements.

3. The period of decreasing warmth: the stage of decrease of characteristic trees of the warm period and the appearance or the return of the predominant forest constituents of the present day.

A resent summary by Godwin (1934) of pollen investigations in the British Isles corroborates the von Post interpretation with a reversion in the last phase.

The past climatic changes as inferred from the results of pollen analyses (Fig. 5) would appear to be nearly the same as von Post except in modern age.

F) Climatic Changes in North America compared with the Writer's results

In North America, Hansen (1942, 1943 a, 1943 b, 1948), Fuller (1927, 1935), Potzger and his collaborators (1932, 1942 a, 1942 b, 1943, 1944, 1947, 1948 a, 1948 b), and Sears (1931, 1932, 1933, 1935, 1938) are the principal researchers by pollen analysis.

Of these investigations the studies by Sears seem the most important in that not only the examined cases were numerous but also the fields of survey were vast. He concluded on the climatic changes in North America as follows: increasing warmth---maximum warmth---decreasing warmth. But he (1935) recognized that numerous complications ezist to interpret the climates of the second and the third and reserved judgment until the data are more complete.

These results in North America approximately accord with the result of the writer except in modern age.

6. Conclusion

1. From the results of the pollen analyses of Yashimaga-hara bog, the writer determined the forest succession and estimated the climatic changes as shown in Table 9.

2. Pollen analytical studies of Oomine-numa bog yielded results indicating the forest succession and corresponding climatic changes as shown in Table 10.

3. Yashimaga-hara bog consists of peat layers 8.05 m deep, of which the main constituent plant is *Sphagnum*. The absence of volcanic ashes in the peat layers might indicate that the plant communities in this region would have persisted for a long period without suffering from destructive action of volcano, depositing peats continuously since the beginning of the bog formation.

The pollen analyses of Oomine-numa bog showed the existence of a more complicated form of forest succession than that of Yashimaga-hara bog. In place of the upper and lower *Picea* periods of the latter, there are *Abies* and *Fagus-Ulmus* periods in the cold ages of Oomine-numa bog. The altitude of Oomine-numa bog is not so high as that of Yashimaga-hara bog and consequently the forest succession of the former is more complicated than that of the latter. However the general course of the climatic changes in Oomine-numa bog as approached from the forest succession seems to be nearly the same as that of Yashimaga-hara bog (Fig. 5).

Table 9. Forest succession and climatic change at Yashimaga-hara bog.

Depth of peat layer, cm	Forest succession	Climate
0–100	*Pinus* period	Rather warm
100–310	Upper *Quercus* period	Warm
310–410	Upper *Picea* period	Cold
410–640	Lower *Quercus* period	Warm
640–805	Lower *Picea* period	Cold

Table 10. Forest succession and changes of climate at Oomine-numa bog.

Depth of peat layer, cm	Forest succession	Climate
0–120	*Pinus* period.	A little warm
120–220	Upper *Fagus* period	A little cold
220–290	*Alnus* period	A little cold
290–370	*Abies* period	Cold
370–520	Lower *Fagus* period	A little cold
520–650	*Fagus-Quercus* period	A little warm
650–860	*Fagus-Ulmus*	Cold

4. In several bogs (1000～1500 m above the sea level) of the central part of Japan, the writer measured the rate of peat growth by virtue of volcanic ash layers interposed in the peat layers and the time of eruptions of volcanoes which are considered as responsible for these ashes. From about sixty of such estimations the rate of 1 mm/year was obtained. According to this rate of peat growth, the time required for the peat formation of Yashimaga-hara and Oomine-numa can be estimated as about 10,000 years.

5. The climatic changes obtained from the study of pollen analyses in Yashimaga-hara and Oomine-numa bogs may be summarized as follows: about 10,000 years ago when Yashimaga-hara and Oomine-numa bogs began to be formed, the climate was cold. Thereafter the climate became gradually warmer with the warmest peak at about 6,000 years ago. Following this from 4,000 to 3,000 years ago the climate became again cold. Since then the climate has been becoming warmer (Fig. 5).

6. The past climatic changes in the central part of Japan as inferred from the results of pollen analyses would appear to be nearly the same as those in Northern Europe and in North America except in the modern age.

7. Summary

1. The writer has performed pollen analyses in several bogs in the central part of Japan. Among them the bogs of Yashimaga-hara and Oomine-numa possess peat layers of more than 8 m in depth, and probably the plant com-

munities of these bogs were uninterrupted by volcanic activities which happened to do so in several bogs of Japan. By the pollen analyses of these peat layers results were obtained which enabled to clarify the forest succession and to infer the climatic changes (Table 9, 10, Fig 5).

2. The writer determined the age of peat layers by the use of volcanic ash layers inserted between peat layers. Thus the date of the formation of volcanic ash layers can be known from the record of the eruption in the literature. From the number of years which have elapsed from the eruption till now and the thickness of peat layer the rate of the peat growth was calculated. From the results obtained it appears probable that the rate of peat growth in the bogs in the central hill districts of Japan, lying about 1000~1500 m above sea level, may be about 1 mm/year. Accordingly the time required for the formation of Yashimaga-hara and Oomine-numa bogs may be computed to be about 10,000 years.

The climatic changes approached from the study of pollen analyses of these bogs may be considered as follows (Fig. 5): about 10,000 years ago when Yashimaga-hara and Oomine-numa bogs were supposed to appear first, under cold climatic condition. Then the climate became gradually warmer until at about 6000 years ago. Following this from 4,000 to 3,000 years ago, the climate was again cold, and thereafter the climate has been becoming warmer.

3. The results of the pollen analytical studies obtained by the writer might indicate that the climatic changes in the past of the central part of Japan were nearly in accord with those in Northern Europe and in North America except in modern age.

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Studies on the Species Differentiation in the Section Tuberarium of *Solanum*. IV. Cytological Behavior of the F_1 Hybrids from *S. tuberosum* × *S. saltense* (4x), with Special Remarks on the Polyploid Nature of *S. tuberosum*.*

By

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Introduction

For our knowledge on the origin and evolutionary process of *S. tuberosum* and for the theoretical basis of potato breeding, it is very important to investigate the nature of polyploidy of this species. This problem has already been discussed from cytological, genetical, morphological and physiological points of view, by many workers (Fukuda, 1927; Longley & Clark, 1930; Meurman & Rancken, 1932; Ellison, 1935, 1936; Baylis, 1936; Lunden, 1937; Ivanovskaja, 1939; Cadman, 1942, 1943; Lamm, 1945; Krantz, 1946; Thomas, 1946; Swaminathan & Howard, 1953; Swaminathan, 1954; Hawkes, 1956).

In this field, Swaminathan's works (1954) should be noted above all. He, who has comprehensively reviewed the literature concerned with this subject and carried out cytogenetic studies of *S. tuberosum* and its relatives, concluded that "*S. tuberosum* probably arose as an autotetraploid."

Polyploid nature of *S. tuberosum*, however, seems to be too complicated to explain only from this view. In order to bring this problem to a reliable conclusion, a further detailed study must be done by means of the following two ways: directly, to observe the meiotic behavior and the fertility in some haploid plants of this species, and indirectly, to infer the pairing behavior in the two gametic sets of *tuberosum*-chromosomes, from several F_1 hybrids between *S. tuberosum* and autotetraploid *Solanums* which were induced from diploid species more remotely related to *S. tuberosum*, such as those belonging to the series *Commersoniana*. The former work has been tried earlier by Ivanovskaja (1939) and the works similar to the latter have already been done by Oppenheimer (1933), Propach (1938), Stelzner (1943) and Prakken & Swaminathan (1952).

In the present work, the tetraploid F_1 hybrids from the cross *S. tuberosum* × induced autotetraploid *S. saltense* were studied in detail by the latter way, and the results are given in this paper.

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Material and Methods

Three F_1 hybrids (2n=48) obtained from crossing *S. tuberosum* L. (variety Deodara) with induced autotetraploid *S. saltense* Hawkes were used for the present work. All of the three hybrids were ascertained to be tetraploid plants having 48 somatic chromosomes. Of both the parent species, the former was supplied from the Potato Experiment Station at Shimamatsu, Hokkaido and the latter whose original diploids have been sent through the Inter-regional Potato Introduction Station at Sturgeon Bay, Wisconsin, is what its chromosomes were doubled by colchicine treatment at our laboratory. In the cross, ten pollinated flowers of *S. tuberosum* used as the female parent produced six berries, the average number of seeds being 73.7 per berry. The hybrid seeds germinated very well.

For observations of the meiotic configurations of the pollen mother cells and for analysis of the pollen grains, the methods reported previously by Matsubayashi (1955 a) were employed.

Results

Diakinesis and Metaphase I: Results that the meiotic configurations at these stages were in detail analysed are given in Table 1. In tetraploid

Table 1. Chromosome associations at diakinesis and M-I in the hybrids.

Stage		No. of cells studied	Frequency per cell of			
			IV	III	II	I
Diakinesis	Mean	12	1.17	1.25	18.25	3.08
	Range		0~2	0~3	15~23	0~6
Metaphase	Mean	75	0.60	1.29	19.00	3.72
	Range		0~2	0~4	11~24	0~13

materials of *Solanum*, secondarily associated groups of two bivalents are liable to be sometimes mistaken for true quadrivalents. In order to avoid such mistake, the authors observed the chromosome associations at diakinesis as a criterion for judging the meiotic configurations at the first metaphase.

At diakinesis in the hybrids, the mean frequency of pairing was $1.17_{IV}+1.25_{III}+18.25_{II}+3.08_{I}$ and two bivalents were usually attached to the nucleolus. The meiotic configurations at the first metaphase were nearly similar to those at diakinesis, while there was a little reduction of the quadrivalent frequency, showing $0.60_{IV}+1.29_{III}+19.00_{II}+3.72_{I}$ (Figs. 1 and 2).

Similar tetraploid material have been studied by Oppenheimer (1933), Propach (1938) and Prakken & Swaminathan (1952) in the F_1 hybrids from *S. chacoense* × *S. tuberosum* and *S. tuberosum* × *S. chacoense* respectively. These mean pairing frequencies were found by the former to be $0.56_{IV}+22.82_{II}+0.12_{I}$ and by the latter $1.15_{IV}+21.46_{II}+0.56_{I}$. The pairing behavior in the present materials

is thus fairly different from that in their ones, in showing the occurrence of trivalent formation and the increased frequency of univalent chromosomes. As seen in Table 2, the frequency of univalents varied widely from 0 to 13 with a mean of 3.72 and that of bivalents ranged also widely from 11 to 24 with a mean of 19.00. Among these bivalents, each of about six was very loosely

Table 2. Frequency of univalents and bivalents at M-I in the hybrids.

	No. of cells studied	Number of univalents or bivalents per cell																	Total	Mean
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Univalents	75	3	8	10	17	18	8	4	1	4				1	1					279	3.72
Bivalents loosed	75			3	6	11	12	13	11	11	6	1	1							446	5.95
Bivalents closed	75								2	1	4	3	5	14	14	13	7	5	7	979	13.05

paired by single terminal chiasma and appears to be able to change without so difficulty into two univalents under occasional conditions. These phenomena should be noticed for considering affinity between the two gametic sets of *tuberosum*-chromosomes in the hybrids. Most of multivalent associations were of N-, U- and O-shapes in the quadrivalents and of V-, P-shapes and chain type in the trivalents (Fig. 1).

Anaphase I and Telophase I: The chromosome behavior was usually regular throughout the first anaphase and telophase in most cells, but in some cells (about 20%) the lagging chromosomes ranging from 1 to 4 were found (Tab. 3 and Fig. 3). The laggards that failed to be included in both of the daughter nuclei seem to form micronuclei with different size at the second telophase.

Table 3. Frequency of laggards at A-I and T-I in the hybrids.

	Number of laggards per cell					Total
	0	1	2	3	4	
Freq.	90	29	19	8	5	151
%	59.60	19.21	12.58	5.30	3.31	

Metaphase II. The chromosome numbers per plate at the second metaphase were counted in 136 plates, and the data are shown in Table 4. As will be seen from the table, numerically balanced plates with 24 chromosomes amounted to 49.26% of the cells studied in the hybrids. This value is almost similar to those of Oppenheimer (1933) and Propach (1938), who have counted 48.5% and 49–66% respectively in the tetraploid hybrids between *S. tuberosum* and *S. chacoense*, but far lower than that (84%) observed by Prakken & Swaminathan (1952) in the similar hybrids. The chromosome numbers at the second metaphase should theoretically show a symmetrical distribution on either side of the mean, whereas in this case there was a deviation towards the left side of the

Table 4. Frequency of chromosome numbers at M-II in the hybrids.

	Chromosome number per plate								Total
	20	21	22	23	24	25	26	27	
Freq.	1	3	16	22	67	19	6	2	136
%	0.74	2.21	11.76	16.18	49.26	13.97	4.41	1.47	

mean. This deviation from the expected random distribution is certainly due to the occasional loss of chromosomes from some of the nuclei (Figs. 3 and 4). Second division restitution was found in some cells.

Pollen: The well stained grains with normal form were grouped by their size into three classes; *i.e.* G, M and S having diameter of 35–42, 26–30 and 20–24 in μ respectively (Tab. 5). Perhaps, the giant pollens may have been

Table 5. Pollen analysis in the hybrids.

	Stainable					
	Normal form*			Abnormal form	Empty	Total
	G	M	S			
Freq.	57	684	40	85	829	1695
%	3.36	40.35	2.36	5.01	48.91	
	46.07			53.92		

*G, M and S are distinguished by their size from one another, their diameters being 35–42, 26–30 and 20–24 in μ respectively.

caused by double restitutions in the first and the second meiotic divisions (Fig. 5). The total percentage of these pollen grains amounted to about 46, showing lower value than in the tetraploid hybrids from *S. tuberosum* × *S. chacoense* studied by Prakken & Swaminathan (1952).

Discussion

From that the F_1 hybrids were apparently intermediate between both the parents in many morphological characters, it is not doubtful that 48 somatic chromosomes of these plants would consist of 24 chromosomes from each of the parent species.

For considering on a mode of chromosome associations found in the hybrids, the two chromosome sets from *S. tuberosum* are signified as T_1T_2 and those from *S. saltense* (4x) as SS. Thus, chromosome constitution of the hybrids would be formularized as T_1T_2SS. Since the induced tetraploids of *S. saltense* have been found by Matsubayashi (1955 b) to have a significantly higher multivalent frequency in comparison with the other allotetraploid species of *Solanum*,

it is enough considered that the two sets (SS) of *saltense*-chromosomes in the hybrids are homologous to each other. On the other hand, Swaminathan (1953) suggested that a high trivalent frequency in some tetraploid hybrids from the triple cross (*S. stenotomum* × *S. saltense*) × *S. tuberosum* indicates a lower affinity between the chromosome sets of *saltense* and *tuberosum* as compared with *stenotomum* and *tuberosum*.

In the present hybrids, therefore, the most likely explanation of the paired chromosomes including the multivalents seems to be that 12 paired chromosomes are usually formed by autosyndesis between S and S, and the remainings between T_1 and T_2. It is also very probable to consider that the univalents are derived from occasional failure of pairing between T_1 and T_2 and the multivalents are formed among T_1, T_2, S and S. Oppenheimer (1933), Propach (1938) and Prakken & Swaminatnan (1952) have alike considered autosyndetic pairing as a interpretation on the chromosome association found in some tetraploid hybrids between *S. tuberosum* and *S. chacoense*.

Thus, pairing potentiality between T_1 and T_2 may be indicated by a generalized meiotic configuration $10_{II}+4_I$, 10_{II} of which were calculated by adding the complements taking part in the multivalents to the remaining 7 bivalents. This pairing potentiality, moreover, appears to be not so strong, because it is presumed, from both frequencies of the loosed bivalents and the univalents (Tab. 2), that the paired chromosomes are made to easily change into unpaired ones by certain conditions.

Considering such facts, it is inferred that, although the two genomes in the gametic sets of *S. tuberosum* are capable of forming 12 bivalents by preferential pairing, true affinity between them is not so much great. This induces the authors to regard that *S. tuberosum*, so far as its variety Deodara is concerned, has the nature of more allotetraploidy than autotetraploidy, implying that, as Hawkes (1956) has suggested, this species "may have been formed as an amphidiploid hybrid between two species whose genomes were not quite identical."

The view mentioned above is not in agreement with Swaminathan's conclusion (1954) that "*S. tuberosum* seems essentially to be an autotetraploid," even if he restated additionally that "though the current commercial varieties can be considered as segmental allotetraploids." This would be supported, however, by the conclusion of Ivanovskaja (1939) that *S. tuberosum* is not an autotetraploid owing to the reason that a haploid plant of this species is almost completely sterile though it can often form 12 bivalents, and also by the observations of Lamm (1945) and Matsubayashi (unpub.) that the variety Deodara has a significantly lower multivalent frequency than in some induced autotetraploid *Solanums*.

Summary

(1) Meiosis was observed in the tetraploid F_1 hybrids from *S. tuberosum* (var. Deodara) × induced autotetraploid *S. saltense*. These hybrids were found to show the mean pairing frequency of $0.60_{IV}+1.29_{III}+19.00_{II}+3.72_I$ at the first

metaphase, the balanced plates amounting to about 49% of the cells observed at the second metaphase and 46% stainable pollen on the average.

(2) From the most possible mode of chromosome pairing in the hybrids, it was inferred that true affinity between the two gametic chromosome sets of *S. tuberosum* is not so much high, suggesting that this species may have originated as an allotetraploid.

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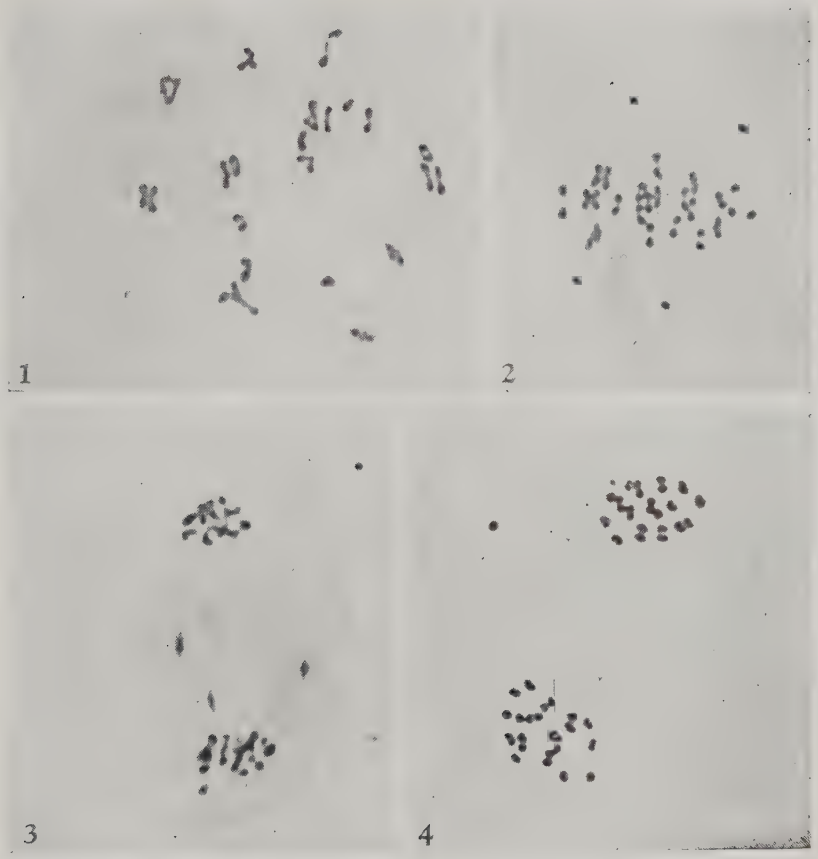

Meiosis and pollen grains in the F_1 hybrids, *S. tuberosum*×*S. saltense* (×4).

Fig. 1. M-I, $2_{IV}+2_{III}+16_{II}+2_I$. Fig. 2. M-I, $1_{III}+20_{II}+5_I$. Fig. 3. T-1, showing 3 laggards. Fig. 4. M-II, 24-1-23. Fig. 5. Pollen grains, especially the arrows show giant ones.

(Figs. 1-4: ca.×2000. Fig. 5: ca.×150)

Studies on Growth in the Axial Organs of Bean Seedlings. I. Chemical Changes in Growing Tissues.

By

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Introduction

Since Sachs' classical observation (cf. 4) that in bean roots the elongation rate is low both at the extreme tip and the basal region and the highest rate is seen in the subapical region, the growth patterns in the axial organs such as stems and roots have been studied by many workers (cf. 7).

In these years biochemical analyses of the growth process of germinating seed embryos of a bean, *Vigna sesquipedalis* have been one of the main concerns of this laboratory. Oota et al. (21) have compared the chronological changes in chemical constituents of the catabolic organ, i. e., the cotyledons, with that of the anabolic ones such as the plumules, the hypocotyls, etc. during the germination stages.** Special attention has been paid on the mechanism of accumulation of protoplasmic protein ("the PP–pattern of growth") in the stem portion (19, 20, 21, 22). In most of the previous studies in this laboratory, however, each embryonic organ was examined as a whole and the local and chronological change of the growth patterns in the respective organs remained to be analyzed.

In the present investigation several zones of different growth activities were isolated from the etiolated hypocotyl axes and their chemical compositions were analyzed on the cell basis to gain some insight chiefly in chemical aspects of the growth pattern in the axial organs. Energetics of the growth process will be dealt with in the 2nd paper of this series.

Materials and Methods

Experimental materials: Seeds of *Vigna sesquipedalis* (the 1955 harvest) purchased at market and stored in a dark desiccator for about one year were used. For germinating the seed methods similar to those of Oota et al. (21) (at 30°C, in the dark) were employed. Seedlings of 2, 3 and 4 days of age were used; the length of the hypocotyls were about 4 cm, 9 cm

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** The difinition of the germination stage is seen in Oota et al. (21).

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and 13 cm, respectively. In order to see the elongation pattern in the hypocotyls printing ink lines at 2 mm intervals were marked on the surfaces of the 2– and 3–day-old hypocotyl-axes from the cotyledon attachment downwards by mean of a home-made marking instrument under red light. Changes in the mark intervals after one more day cultivation are shown in Fig. 1. In younger hypocotyls (2– to 3–day-old) the maximum growth zone was seen at the subapical part, an almost similar growth pattern to that known in the roots (cf. 4). In the 3– to 4–day-old hypocotyls the pattern was modified by an acropetal migration of the active zone along the hypocotyl axis (cf. 21). Three zones (A–, B– and C–zones, each 5 mm long) of various growth activities were selected from the 3–day-old hypocotyls as shown in Fig. 2. After a day, or in the 4–day-old hypocotyls, A–, B– and C–zones were 25 m, 10 mm and 5 mm long, respectively. In addition to these hypocotyl segments the uppermost 2 mm portions of the 2–day-old hypocotyls were also picked out (Fig. 2). The portions were 5 mm long on the next day to be above mentioned A–zone of the 3–day-old hypocotyls (Fig. la). Seven stem segments in all, i. e., 2–, 3–, 4–A; 3–, 4–B and 3–, 4–C,* were cut off to be used for various morphological as well as chemical analyses. In other words, the starting and the terminal states of four phases of the hypocotyl growth were examined, respectively:

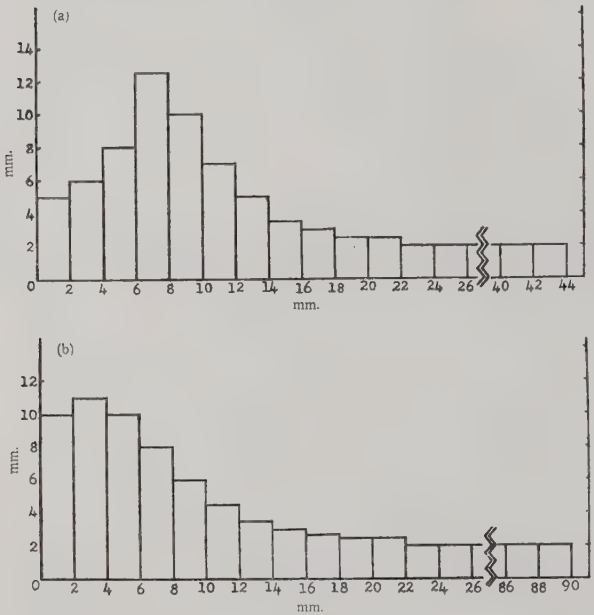


Fig. 1. The growth (elongation) parttern of the bean hypocotyls.
(a) Ink-lines were marked at intervals of 2 mm on the 2–day-old hypocotyls (44 mm long) (the abscissa); Intervals between two neighbouring lines were meansured on the next day (the ordinate).
(b) Ink-lines were marked at intervals of 2 mm on the 3–day-old hypocotyls (90 mm long) (the abscissa); Intervals between two neighbouring lines were measured on the next day (the ordinate).

* Numerals denote the ages (in days) of the hypocotyls from which zones are excised, e. g., 2–A means A–zone of the 2–day-old hypocotyls.

Phase I, the 1st slow growth (2-A → 3-A), Phase II, the most rapid growth (3-A → 4-A), Phase III, the 2nd slow growth (3-B → 4-B) and Phase IV, no growth (3-C→4-C).

Cell number: Longitudinal and transversal sections were made from respective zones isolated (5 to 10 samples were used for each zone) with a microtome, and cells contained in each section were counted under a microscope. The total cell number of a zone was obtained by multiplying the maximum cell number found in the longitudinal cuts by the transversal cell number.*

Cell volume: Ten to 20 pieces of respective zones were immersed in water in a 10 ml calibrated cylinder, and the rise of water level was read as the total volume of pieces immersed. Cell volume=zone volume/total cell number of the zone obtained above.

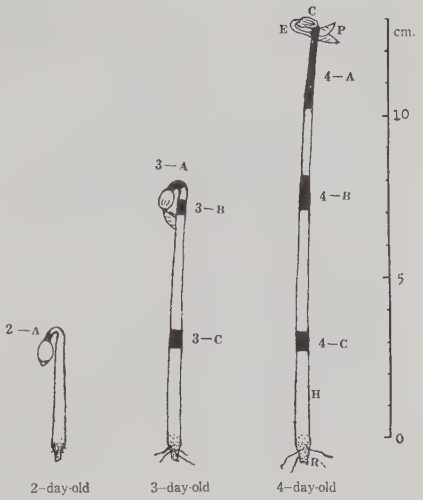


Fig. 2. Diagramatic representation of the hypocotyls; three (A-, B- and C-) zones used are shown.

P: plumules C: cotyledon
E: epicotyl H: hypocotyl
R: radicle

Fresh weight, dry weight and water content: Twenty to 30 pieces of respective zones were placed in a weighing bottle and the fresh weight was estimated. They were then dried at 100°±5°C until constant weight (the dry weight) was attained. The difference between the fresh and the dry weights were regarded as the water content of the tissues.

Protein nitrogen: Zones to be examined (40–50 pieces) were homogenized in a mortar and treated with 10% trichloracetic acid (TCA). The precipitate was washed twice with 5% TCA. The total nitrogen content of the precipitate (protein N) was estimated by a modified Levy-Palmer's method (31). Coefficient 6.2 was used in the calculation of protein amount.

Non-protein nitrogen: The isolated zones (10–20 pieces) were digested with conc. H_2SO_4 and H_2O_2, and the total (protein+non-protein) N was estimated with the above mentioned method. Non-protein N=total N − protein N. The non-protein N content multiplied by 6.2 was provisionally considered to be the amount of non-protein N compounds.

Reducing and non-reducing sugars: Homogenates of zones (10–20 pieces) were extracted with 80% ethanol at 80°–85°C. After alcohol was evaporated,

* In relation to the transversal cell number little zonal difference was found in the hypocotyls used (see below).

the extract was clarified with neutral lead acetate, potassium oxalate and Amberlite IR-150, and, after neutralized, was estimated for reducing sugars by the Somogyi's method (27). The increase in reducing power by an acid hydrolysis (heated for 30 min. in a boiling water-bath in presence of 0.05 % HCl) was considered to come from non-reducing sugars. Sugar content will be expressed as glucose equivalent.

Starch: Isolated zones (50–100 pieces) were homogenized in a mortar and assayed for starch by the method of Pucher et al. (24). Starch content will be expressed as glucose equivalent.

Cell wall materials:* Isolated zones (50 pieces) were homogenized in a mortar and washed repeatedly with cold and hot water. The residue was dried at 100°±5°C until constant weight was attained. The dry matter obtained was considered to be cell wall materials.

Osmotic concentration: This was estimated by the incipient plasmolysis method using mannitol as a plasmolyzing agent, and will be expressed in terms of mannitol concentration (25).

Cell wall extensibility: The methods of Ruge (25) and Ordin et al. (23) were used with a slight modification. By means of a razor blade a strip of epidermis extending over total segment length was peeled off from each zone to examined and its length (L_1) was measured under a microscope with a decimillimeter cross-stage. The remaining hypocotyl segment was floated on distilled water at 30°C for 4 hours. The epidermis was again stripped and its length (L_2) was measured. Then the segment was immersed in a hypertonic mannitol solution (0.70 mol.). After a 2-hour-incubation, the length (L_3) of epidermis was measured in the same way as above. The differences L_2–L_3, L_3–L_1 and L_2–L_1 were regarded as the elastic, the plastic and the total extensions, respectively.

Each figure shown below is the mean of at least five separate estimations.

Results

1. Morphological analyses

Cell number

Table 1 shows the cell number in various zones as estimated for various

Table 1. The number of cells contained in various
Ep: epidermis Co: cortex E:

Tissue \ Age (days) / Zone	2	
	A	A
Ep	42,000— 51,920	58,000— 68,200
Co	165,000—200,600	253,000—283,000
En	172,500—208,000	330,000—384,000
Pi	19,500— 28,500	25,500— 36,200
Total	399,000—489,020	666,500—771,400
Mean	444,010	718,450

* The cell number of 4-C was not studied because of a partial histolysis

* The data on cell wall materials were kindly furnished by A. Mizuochi and Y. Morimoto of this laboratory.

component tissues, i. e., epidermis, cortex, "endodermal region" and pith (Fig. 3). Owing to a partial histolysis, the cell counting for 4–C was impossible. It will, however, reasonably be considered that the cell number of 4–C would practically be equal to that of 3–C (see below). No significant difference in cell number is found in the transversal sections of different zones examined. It is also seen in this table that the cell number is maintained nearly constant throughout Phase II, Phase III or Phase IV (the mean values in round numbers, 720,000 for 3– and 4–A, 220,000 for 3– and 4–B, and 120,000 for 3–C and also probably for 4–C). It has been pointed out that in the later germination stage the growth of the hypocotyl does not involve any cell division but only cell elongation (18). On the contrary, a distinct increase in cell number is seen in Phase I (from 440,000 in 2–A to 720,000 in 3–A). This coincides well with the conclusion of Morimoto (unpublished) who has found a greater elongation rate for 1– or 2–day-old hypocotyls as a whole than that for the average epidermal cell contained in the tissues. The increase is remarkable especially in "the endodermal region." Microscopically, however, no cell division figure has been detected.

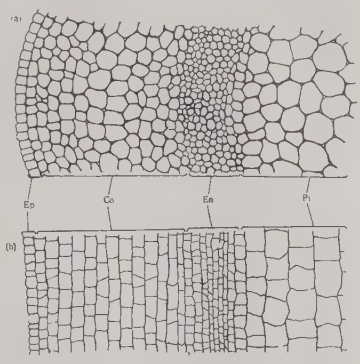


Fig. 3. The miroscopic structure of the hypocotyl as observed for the 3–A zone.
(a) transversal section
(b) longitudinal section
Ep: epidermis Co: cortex
En: "endodermal region"
Pi: pith

zones of the bean hypocotyls of various ages.
"endodermal region" Pi: pith

3		4*	
B	C	A	B
18,000— 22,000	10,000— 13,200	56,000— 66,000	18,000— 22,000
72,600— 82,600	44,000— 54,200	264,000—308,000	76,400 - 80,240
105,000—128,000	52,500— 64,000	322,500—370,000	97,500—120,000
8,400— 11,400	4,200— 6,080	25,500— 36,200	9,000— 12,160
204,000—244,000	110,700—137,480	668,000—781,000	200,900—234,400
224,000	124,090	724,500	217,650

in the pith portion.

Cell volume

The results of the cell volume estimation are shown in Fig. 4. It is

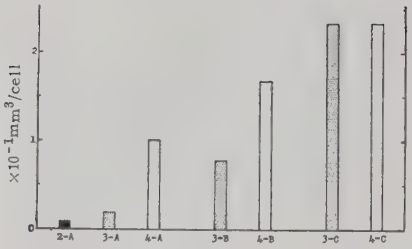


Fig. 4. The mean volume of cells in various zones of the bean hypocotyls.

evident that the cell volume is generally smaller in the upper part than in the lower one. In Phases II, III, and IV, although the cell volume changes nearly in parallel with the zone length (cf. Fig. 2), the rate of increase in cell volume is slightly higher than that in total zone length. Accordingly a little lateral expansion of cells is to occur simultaneously with the longitudinal one. The increase in cell volume is also observable in Phase I.

2. Chemical analyses

The contents of several chemical constituents, i. e., proteins, non-protein nitrogen compounds, soluble sugars, starch, cell wall materials and water were estimated. It should be noted that the dry weight of a cell is always nearly evual to the sum total of the weights of above chemical constituents estimated for the cell (compare Table 5 with Fig. 6). Hence the content of fresh materials of the cell may reasonably be itemized as follows:

fresh materials { water
dry materials { proteins
non-protein N compounds
soluble sugars
starch
cell wall materials

Fresh weight, dry weight and water content

The results of the estimation of fresh weight, dry weight and water content are shown in Figs. 5, 6 and 7, respectively. Regional and chronological changes in fresh weight as well as in water content per cell are quite similar to those in cell volume (cf. Fig. 4). It is clearly demonstrated

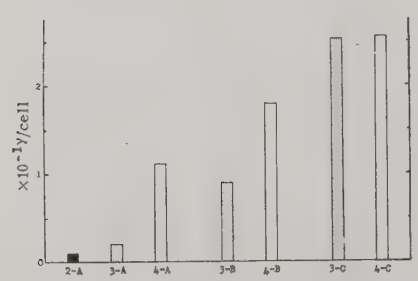


Fig. 5. The mean fresh weight of cells in various zones of the bean hypocotyls.

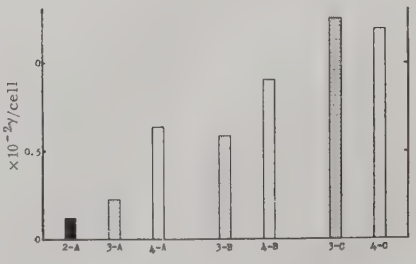


Fig. 6. The mean dry weight of cells in various zones of the bean hypocotyls.

that almost all (90–95 %) of the fresh weight increase is, as expected, due to the accumulation of water. The rate of increase in dry weight does not always go in parallel with that in water content. The difference is greatest in Phase II, where the rate of dry weight increase is about half as much as that of water content increase.

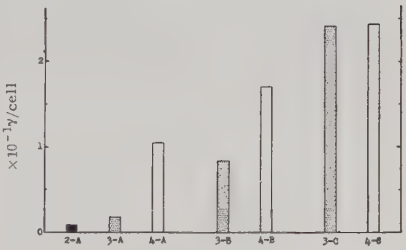


Fig. 7. The mean water content of cells in various zones of the bean hypocotyls.

The percentage water content, i.e., water content/fresh weight × 100, of the hypocotyl cell (Table 2) is found to increase distinctly with the cell elongation, e. g., in Phase II it increases from 89 % up to 94 %. Kramer and Wiebe (17) using barley and pine roots have also found that percentage water content is 89 % at the tip (meristematic) region and 93–94 % at the subapical (elongation) one. It appears to be a general trend in axial organs that percentage water content of rapidly elongating part is higher than that of meristematic one.

Table 2. Chronological change in percentage water content* of various zones of the bean hypocotyls.

Age (days) \ Zone	A	B	C
2	87.5	--	
3	89.2	93.5	95.0
4	94.0	95.0	95.4

* $\frac{\text{water content}}{\text{fresh weight}} \times 100$

The above results would support a common view that the elongation mechanism will be connected predominantly with the water uptake (cf. 8, 28).

Proteins and non-protein nitrogen compounds

The protein N content per cell of respective zones are shown in Fig. 8. The changes in content are remarkably different from those in cell volume (cf. Fig. 4) or in water content (cf. Fig. 7). Thus the protein content reaches a maximum before the elongation is finished. In the 4-day-old hypocotyls regional difference is hardly seen. Although in the early stage of growth, i. e., Phases I and II, elongation is accompanied by protein accumulation, in Phase III no protein accumulation occurred in spite of still continuing elongation. A little decrease in protein is observed in Phase IV. And the rate of protein accumulation is found to be always very much lower than the rate of water uptake. Oota et al. (21) have found that in the bean hypocotyl as a whole the protein content rises up until the middle

of the germination stage and thereafter the protein level is maintained unchanged. The present results would suggest that this apparent stop of accumulation may only reflect a balance established between the increase in protein content in the upper part and the decrease in the lower part of the hypocotyl.

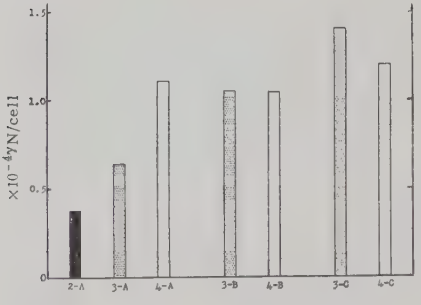


Fig. 8. The mean protein nitrogen content of cells in various zones of the bean hypocotyls.

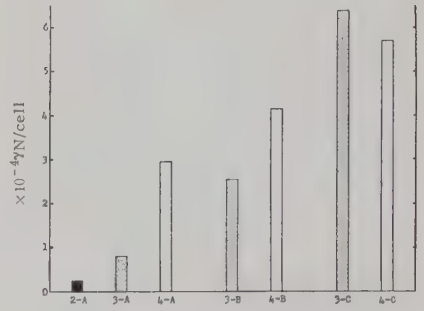


Fig. 9. The mean non-protein nitrogen content of cells in various zones of the bean hypocotyls.

Blank and Frey-Wyssling (2), Chao (9), Baldovinos (1) and Brown et al. (6) have described cell elongation accompanied by protein accumulation, the rates of these two processes being greatly different from each other. On the other hand, Jensen (13) and Schumacher and Matthaei (26) have observed elongation without simultaneous protein accumulation. The relationship between elongation and protein accumulation may vary with the age of tissues. Thus in the present materials, elongation in early germination stage (or in the early growth phases) involves protein accumulation but does not in the later one (or in the later growth phases). Naturally the stop of protein accumulation or even the decrease in protein content does not immediately mean the stop of protein synthesis itself. Chibnall and Wiltshire (10) and Yemm and Willis (33) have shown that N^{15} can still be incorporated into protein of runner bean leaves and barley roots when the total protein content is decreasing. Accordingly it is possible that some protein synthesis is proceeding in the hypocotyl in the later germination stage where no net formation occurs.

The change in non-protein N content per cell is illustrated in Fig. 9. According to Yamamoto (32) of this laboratory non-p otein N compounds in the bean hypocotyl are mainly consisted of amino acids togther with a little amides, chiefly asparagine. The changes in non-protein N content is quite different from that in protein N content (cf. Fig. 8); non-protein N still continues to accumulate after protein N stops to increase. It is noted that the non-protein N content changes nearly in parallel with the water content (cf. Fig. 7). The non-protein N accumulation, therefore, will presumably be in a close connexion with the water uptake. At Phase

IV, however, a slight decrease in the non-protein N content was seen, which might be due to the upward translocation of it in the stem axis (cf. 20, 32). A gradual increase with the advance of growth phase in the ratio non-protein N to protein N contents, is shown in Table 3.

Table 3. Chronological change of the ratio non-protein N to protein N in various zones of the bean hypocotyls.

Age (days) \ Zone	A	B	C
2	0.55	—	- -
3	1.34	2.42	4.53
4	2,66	3.97	4.75

Soluble sugars and starch

The sugar content (Fig. 10) runs nearly parallel with the water content (cf. Fig. 7). This would imply that the soluble sugar accumulation may also be linked with the water uptake. As the water uptake stops (Phase IV) the sugar content decreases distinctly. The sugars lost would have been partly transported upward to the growing regions (cf. 20), partly converted into cell wall materials (cf. Fig. 12) and partly respired*.

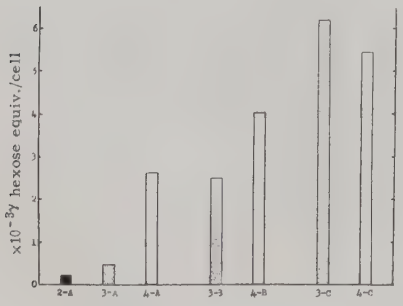


Fig. 10. The neam soluble sugar content of cells in various zones of the bean hypocotyls.

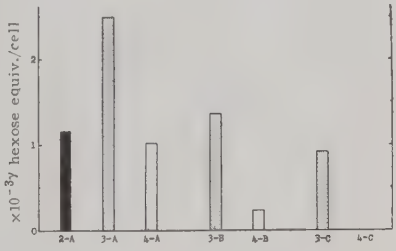


Fig. 11. The mean starch content of cells in various zones of the bean hypcotyls.

A basipetal gradient as to the starch content is seen in the hypocotyl axis (Fig. 11), and, interesting to say, a remarkable amount of starch is accumulated in Phase I (2-A→3-A) and begins to diminish in Phase II

* According to a separate experiment (Izawa, unpublished) the respiratory quotient of the hypocotyl is ca. 1.0 in every growth phase examined. From the amount of oxygen uptaken the respiratory consumption of carbohydrate has been calculated as $2.26\times10^{-4}\gamma$, $4.81\times10^{-4}\gamma$, $6.18\times10^{-4}\gamma$ and $7.16\times10^{-4}\gamma$ glucose/cell/24 hours for Phases I, II, III and IV, respectively.

(3-A→4-A)*. With the hypocotyls of *Vigna sesquipedalis* Mizuochi (unpublished) and Kawamatsu (14) have separately found a fact of similar nature histochemically using the KI–I_2 reagent. The coincidence of the time of starch degradation with that of rapid water uptake (Phase II) may suggest that the former would be related osmotically with the latter. Quantitatively, however, the degradation of starch is too small to cover the actual accumulation of soluble sugars (cf. Fig. 10). Thus the starch loss in Phase II can cover only fourteenth of the sugar gain in the same phase (compared on the hexose basis).

In Table 4 are seen the ratios reducing to non-reducing sugars. It is especially noteworthy that the ratio which has been maintained at an almost constant low level during Phase I, suddenly increases in Phase II where non-reducing sugar is found to be almost wholly lost. Fujii (12) of this laboratory has found that in the bean hypocotyl reducing sugars are consisted chiefly of glucose and fructose and that non-reducing sugar involves practically only sucrose. He has also suggested that, as is known for other plants (cf. 29), sucrose may be the major migrating form of carbohydrate in the bean embryos. The cotyledons will provide the seedling axis with sucrose, which in the axial tissues may be split into gulcose and fructose by invertase (12). If so, the rise of the value of the ratio reducing to non-reducing sugars as detected in the hypocotyl tissues would indicate the rise of invertase activity in the tissues in question. The breakdown of sucrose into hexoses may certainly be an effective way of raising the osmotic concentration. According to Wanner and Leupold (30) and Brown et al. (6) who have worked with the roots of broad bean, the region of the maximum invertase activity is the region of rapid elongation.

Table 4. Chronological change of the ratio reducing sugars to non-reducing sugars in various zones of the bean hypocotyls.

Age (days) \ Zone	A	B	C
2	1.00	—	—
3	1.57	12.7	23.7
4	16.4	24.5	31.8

Cell wall materials

In every phase excepting Phase IV the change in amount of cell wall materials of a cell (Fig. 12) is similar to that in dry weight of the cell

* Oota et al. (21) have stated that the content of water-soluble alcohol-insoluble carbohydrates (tentatively regarded as starch) in the whole bean hypocotyl, gradually increases with the age of the tissues. By means of the present method of starch assay the author (unpulished) has found that the starch content in the hypocotyl passes a peak on the 3rd day of the germination stage. Thus the starch content was 65 γ, 225 γ, 345 γ, 128 γ and 20 γ (hexose equivalent per one hypocotyl) for 0-, 1-, 2-, 3-, 4- and 6-day-old materials, respectively.

(cf. Fig. 6). In Phase II the rate of increase of cell wall materials is smaller than that of cell volume (cf. Fig. 4). It is also noticeable that the cell wall materials still continue to increase but slightly when the tissues cease entirely to expand. This may naturally mean thickening of the wall. There is no simple parallelism between the cell wall materials and the cell elongation.

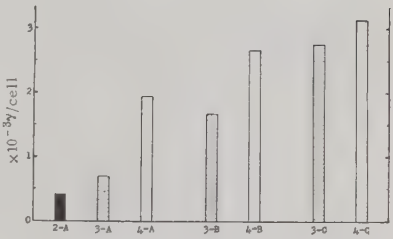


Fig. 12. The mean cell wall material content of cells in various zones of the bean hypycotyls.

In the hypocotyls of *Vigna sesquipedalis* it is known that in the early germination stage the formation of cellulose is less active than that of hemicellulose and the situation is reversed in the later germination stage (21). Morimoto (unpublished) has found a similar pattern along the hypocotyl axis. Thus a predominant formation of cellulose is seen in the basal portion or in the more aged tissues and that of hemicellulose in the upper portion or in the younger tissues.

Composition of dry material

As has been described at the outset of this paper, almost all of the dry matter of the tissues is recovered as proteins, non-protein N compounds, soluble sugars, starch and cell wall materials (Table 5 and Fig. 6). A remarkable fact is shown that the part occupied by high-molecular substances, i. e., proteins, starch and cell wall materials, in comparison with that occupied by low-molecular ones, i. e., soluble sugars and non-protein N compounds, gradually decreases with the age of the tissues. Thus, the amount of the former that occupied about 60–70 % of the whole dry matter of the cells in Phase I, falls to 30 % in Phase IV. It is also shown that in Phase I almost equal amounts of the high-molecular and the soluble substances are accumulated, but later in Phase III the deposition of the

Table 5. Composition of dry matter in various zones of the bean hypocotyls of various ages.*

Age (days) / Zone / Cell constituent	2	3			4		
	A	A	B	C	A	B	C
Soluble sugars	0. 022	0. 045	0. 248	0. 620	0. 260	0. 402	0. 545
Starch	0. 011	0. 024	0. 013	0. 009	0. 010	0. 002	—
Protein	0. 026	0. 036	0. 065	0. 087	0. 068	0. 064	0. 074
Non-protein N compounds	0. 015	0. 048	0. 156	0. 394	0. 182	0. 256	0. 352
Cell wall materials	0. 042	0. 069	0. 163	0. 275	0. 195	0. 263	0. 313
Total	0. 116	0. 222	0. 645	1. 385	0. 715	0. 987	1. 284

* Figures in $10^{-2}\gamma$/cell are shown.

latter is accelerated and the increase of soluble substances amounts up to ca. 70 % of the dry weight increase. In this connexion it will be interesting to note a microscopical finding that in the epidermal cells in Phases III and IV marked development of vacuoles are seen, while no visible vacuolization occurs yet in Phase I.

2. Factors controlling water uptake

It is widely accepted that water uptake is a essential requisite for cell elongation (8, 28), and from the aforementioned this appears to be also the case for the present materials. The water uptake may be regulated by several factors, i. e., solute concentration, wall pressure, permeability of protoplasmic membrane and cellular activity connecting with water uptake against concentration gradient (cf. a general discussion of Kramer, 16). As to the so-called active water uptake against concentration gradient unfavorable evidences are accumulating (8, 15, 23, 28). It has also been found separately that the hypocotyl segments (3–, 4–A and 3–B) cannot absorb any water when they are floated on hypertonic (0.45–0.55 mol. mannitol) solutions (Izawa, unpublished). Detailed analyses on the permeability of protoplasmic membrane is remained to be made, but it is likely that the permeability could only alter the rate of water transport but not the equilibrium state of water uptake.

Changes in solute content

As already mentioned, the contents of both non-protein N and soluble sugars change in parallel with the water content, though the rate of solute accumulation is always somewhat smaller than the rate of water uptake. Thus, the solute conceneration of the tissues estimated roughly from the content of water and that of solutes inclusive of inorganic ions* (Table 6) gradually decreases with the advance of growth phase. On the other

Table 6. The concentrations of osmotic active substances contained in various zones of the bean hypocotyls of various ages*.

Age (days) / Zone / Solute	3			4		
	A	B	C	A	B	C
Soluble sugars	0. 12	0. 14	0. 14	0. 14	0. 13	0. 12
Non-protein N compounds	0. 30	0. 26	0. 18	0. 22	0. 18	0. 16
Inorganic ions**	0. 11	0. 07	0. 04	—	—	—
Total	0. 53	0. 47	0. 36	0. 36	0. 31	0. 28

* Figures in mol. are shown.
** Unpublished data of Okamoto.

* Okamoto of this laboratory kindly furnished us with his unpublished data on the inorganic ion concentrations of various zones of the 3-day-old hypocotyls. His estimates include potassium-, sodium-ions and total divalent cations. He has found that the inorganic ion concentration estimated for the 4-day-old hypocotyls as a whole is lower than that estimated for the 3-day-old hypocotyls by 20–30 %.

hand, the osmotic concentration of the epidermal cells of every zone other than 2-A* was estimated directly by the incipient plasmolysis method (Fig. 13). It is seen that the values obtained by these two methods well agree with each other. Along the hypocotyl axis there is present a basipetal gradient of osmotic concentration, i. e., the differences between A- and C-zones are ca.0.2 mol. and ca. 0.07 mol. for the 3- and the 4-day-old hypocotyls, respectively; the highest value being found at the uppermost region. A remarkable drop in the osmotic concentration is observed to occur in Phases II and III. It must be noted that the growth phases with higher solute concentrations do not always show more vigorous water uptake. Separately, using isolated hypocotyl segments, it has been demonstrated that the zones which have lost wall extensibility, i. e., 3-C and 4-B (both tissues have an osmotic concentration of ca. 0.3 mol.) (cf. Fig. 14) can absorb no more water even when they are floated on distilled water and that zones possessing wall extensibility, i. e., 3-B and 4-A (cf. Fig. 14) can absorb water even when they are floated on a water containing sucrose (ca. 0.1 mol.) (relative osmotic concentrations of the tissues are nearly equal to the absolute ones of 3-C and 4-B, respectively). By the way, during the incubation period (4 hours) sucrose can not be uptaken in the tissues, accordingly, the solute accumulation may be one of the factors backing up water uptake but not the exclusive one.

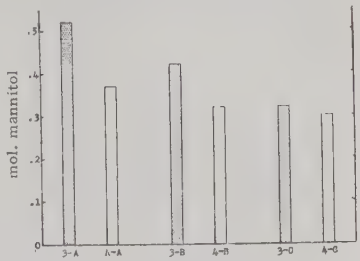
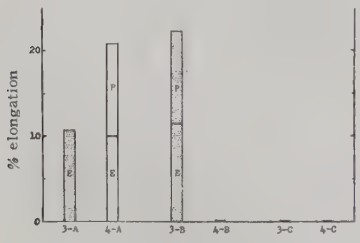


Fig. 13. The osmotic concentration of epidermal cells in various zones of the hypocotyls as estimated by the incipient plasmolysis method.



Eig. 14. The relative wall extensibility of epidermal cells in various zones of the bean hypocotyls.
E: elastic elongation
P: plastic elongation

Changes in wall extensibility

The wall extensibility of epidermal cells of each zone is illustrated in Fig. 14. Regional and chronological changes in wall extensibility are clearly shown to be in parallel with the extent of water uptake. The extensibility in Phase I is only half as much as that in Phase II, and at and after the end of Phase III no extensibility is indicated at all. Concerning the components of extensibility, Phase I found to involve elasticity alone, and Phase II elasticity and plasticity half and half. In other words, the cell

* Because of the absence of visible vacuole, the plasmolysis method was inapplicable for 2-A.

wall expands at first reversibly, then an irreversible extensibility grows up gradually until finally all of the extensibility is lost. It is, therefore, highly probable that the wall extensibility may be the critical factor controlling the water uptake in question.

Discussion

The characteristics revealed for the growth phases examined are summarized as follows:

Phase I. Cell elongation is accompanied by cell division(s). Dry weight increases nearly in parallel with fresh weight, and the percentage water content is maintained almost constant (88–89 %). The ratios reducing to non-reducing sugars and non-protein to protein N in the phase are on the average 1.3 and 1, respectively. Cell wall extensibility is consisted exclusively of elasticity and lacking in plasticity. Starch is accumulated.

Phase II. The most remarkable elongation occurs accompanying no division. Starch begins to disappear. The content of other cellular constituents increases but with the rate considerably lower than the rate of water uptake, the percentage water content being increased up to 94 %. The initial higher osmotic concentration (0.52 mol.) drops to 0.37 mol. by the end of the phase. The ratios reducing to non-reducing sugars and non-protein to protein N rise up 16 and 2.5, respectively. Plastic extensibility being subjoined to the elastic one, the wall is now twice as much extensible as in Phase I.

Phase III. Slow elongation without division. Proteins stop to be accumulated. Cell wall materials, non-protein N compounds and soluble sugars continue to increase in content with the rates lower than that water uptake. The percentage water content rises a little and the osmotic concentration drops down to 0.32 mol. All of the wall extensibility is lost by the end of the phase.

Phase IV. Of the chemical constituents examined only wall materials are slightly accumulated. By the end of the phase the ratios reducing to non-reducing sugars and non-protein to protein N come to 32 and 4.8, respectively. The osmotic concentration drops down to 0.30 mol. No cell wall extensibility is found. Neither elongation nor division takes place.

The changes in content of high-molecular constituents in association with the advance of growth phase will firstly be discussed.

Protein: Working with etiolated bean seedlings, Oota et al.(20, 21, 22) have pointed to a fact that in the hypocotyls protein accumulation occurs only in the first half of the germination stage and in the later half protein begins to deposit in the epicotyls instead (the acropetal migration of "the PP-pattern of growth"). Microsomal ribose nucleic acid (RNA) would have to do with this protein synthesis. It has also been suggested that with age of the tissues the microsomal RNA is liberated into soluble fraction to be transported into the epicotyls. Oota (19) has gone further

to suggest that this decline or stop of protein synthesis in the hypocotyls may be related with the increase in carbon dioxide pressure in the tissues. His examinations on gaseous exchange were done with tissue homogenates. A separate experiment conducted by the present author, however, has shown that aerobic fermentation can hardly take place in the intact tissues in the course of the germination stage (Izawa, unpublished). As pretty high levels of amino acids and amides are maintained in the tissues when the tissues have lost the protein accumulating activity, it is unlikely that the shortage in raw materials will be responsible for this ceasing of protein accumulation. A detailed study now in progress in this labortory on the relation between the protein formation and the state of existence of RNA will provide us with some clue to this problem.

Starch: The starch content was shown to increase temporarily in the initial phase of growth (Phase I). Fujii (12) has suggested that in the hypocotyl in question amylase may not be in action and starch synthesis and degradation would depend largely on phosphorylase action. Soluble sugars are still contained abundantly in the tissues when the starch content is declining, and it has separately been confirmed that only accumulation of sugar but no starch deposition takes place in the isolated 3–B zones floated for 24 hours on the medium containing sucrose or glucose (Izawa, unpublished). In the hypocotyls sucrose can be splitted into glucose and fructose by invertase (6, 12, 30), whose action is generally considered to be irreversible (3). Thus, as to the carbohydrate change in the hypocotyl tissues we can assume a provisional scheme:

$$\text{starch} \rightleftarrows \underset{\uparrow}{\text{sucrose}} \longrightarrow \underset{\uparrow}{\text{glucose}+\text{fructose}}$$

phosphorylase invertase

Noteworthy is that in the present tissues a remarkable increase in the reducing sugar content takes place when starch begins to disappear (cf. Fig. 11 and Table 4). A similar situation has been found in the plumules (Izawa, unpublished). Thus the starch contents were 280 γ, 340 γ and 240 γ and the ratios reducing to non-reducing sugars were 1, 1 and 8, in the 3–, 4– and 6–day-old mate ials, respectively (all carbohydrates were estimated as hexose equivalent per a pair of plumules). It is, therefore, a tentative conclusion that the activity of invertase may determine, in an indirect way, the equilibrium between starch and sucrose.

Cell wall materials: The cell wall is mainly consisted of cellulose, hemicellulose and pectic substances (21). As to the wall production, two remarkable facts should be noted: 1. the production of cell wall can continue even after that of other high polymer constituents of the cell ceases to proceed (the survival of "the CW–pattern of growth," cf. 19 and 21) and 2. the dominancy is shifted from the synthesis of hemicellulose to that of cellulose with the age of the tissues. Regretfully, since too little is known about the mechanisms of production of these wall materials, nothing more can be said here on these interesting facts.

As stated above, cell elongation is practically synonymous with water uptake. It was evidenced that the latter process which will naturally be backed up osmotically by a level of solute concentration may decisively be limited by wall extensibility. Not insignificant accumulation of solutes that was found to occur in the water absorbing regions would perhaps be of physiological significance in the maintenance of a concentration gradient established along the axis.

The extensibility of wall may naturally be related closely to the inner structure of it. According to Frey-Wyssling (11), in corn and oat coleoptiles, microfibrils of the primary wall, that contains hemicellulose as its major constituent, are interwoven in a network texture and those of the secondary, that contains cellulose predominantly, in a parallel texture. He has also stated that the primary wall has a considerable extensibility, while the secondary one which is found only in the basal regions of the tissues has no more of extensibility. Although we have made no examination on the wall structure, it has been demonstrated that the wall extensibility involves elasticity alone in Phase I and both elasticity and plasticity in Phase II, and that all of the extensibility is lost in Phase III. We have also an indication that the cellulose content gradually exceeds the hemicellulose content as the growth phase advances. It will, therefore, be thought that the cells are armed only with the primary wall in Phase I and with the primary and the secondary walls in Phase II. The development of the secondary wall would result in a diminution and finally the disappearance of extensibility of the wall as a whole as actually seen in Phases III and IV. A remarkable increase in wall extensibility occuring Phase II would be interpreted along a suggestion that the secondary wall will not simply be deposited on the surface of the primary one but the framework of the latter will once be softened and extended and therein the secondary wall may be "intussuscept"ed (11). It will certainly be an urgent need to see if the anticipated regional differentiation in the wall structure would actually be present along the hypocotyl axis in question.

A close association of water uptake with respiration has often been demonstrated (cf. 28). Oota (unpublished) has observed that the water uptake of the decotylized bean embryos is entirely inhibited by the addition of minute amounts of respiratory inhibitors or uncoupling reagents. Brauner and Brauner (5) have observed that cell wall extensibility is lower under anaerobic conditions than under aerobic ones. It is noted that in connexion with the auxin action on water uptake the wall extensibility is increasingly attracting our attentions (cf. 8, 23, 28). The relationship between respiration and water uptake is to be elucidated.

The author wishes to express his sincere gratitude to Assist. Prof. Y. Oota, under whose constant guidance this study has been carried out.

Summary

1. Zonal pattern of growth in the etiolated hypocotyls of a bean, *Vigna sesquipedalis*, was investigated. From the 2–, 3– and 4–day-old hypocotyls were excised segments presenting four successive growth phases, i. e., Phase I, the 1st slow growth, Phase II, the most rapid growth, Phase III, the 2nd slow growth and Phase IV, no growth. The growth in Phases II and III involved only elongation but no division.

2. The fresh weight increase was due mainly to water uptake. Dry materials of respective zones were made practically of proteins, non-protein nitrogen compounds, soluble (reducing and non-reducing) sugars, starch and cell wall materials.

3. In the early phases of growth the content of the polymerized matters, i. e., proteins, starch and cell wall materials, increased nearly in parallel with that of the soluble matters, i. e., sugars, amino acids, etc., but in the later phases the accumulation of the soluble matters was chiefly responsible for the dry weight increase.

4. In Phase III the protein level stopped to rise and, thereafter (in Phase IV), it slightly declined.

5. The starch content began to decrease in Phase II. The loss, however, was too small to cover the simultaneous increase in total sugars.

6. The soluble sugars were consisted of nearly equal amounts of reducing and non-reducing sugars in Phase I, and the proportion of reducing sugars was remarkably increased in the later phases.

7. The cell wall materials still increased in Phase IV.

8. The content of soluble sugars together with non-protein nitrogen compounds increased with the age of the tissues and occupied about 70 % of the dry materials in the cells of Phases III and IV. The accumulation of these matters was nearly in parallel with that of water in the cells, and the osmotic concentration of the tissues as estimated by the incipient plasmolysis method was found to be nearly wholly covered by these soluble compounds inclusive of inorganic ions.

9. The cell wall extensibility was the highest in Phase II, where it was twice as high as in Phase I. It was completely disappeared by the end of Phase III. The cell wall extensibility involved elasticity alone in Phase I and fifty-fifty of elasticity and plasticity in Phase II.

10. Physiological meanings of these results were briefly discussed.

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Production of Fungitoxic Substance by Fungi Grown on Media Containing either 2, 4–D or Related Phenoxy Compounds.

By

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Introduction

Gloeosporium olivarum Alm. (causing olive anthracnose) was shown by the author *et al.* to produce a certain antibiotic substance when the pathogen was grown on media supplied with either sodium 2, 4-dichlorophenoxyacetate (2, 4–D) (4, 5, 6, 7), 2–methyl–4–chlorophenoxyacetic acid (MCP), or 2, 4, 5–trichlorophenoxyacetic acid (2, 4, 5–T) (8, 9, 10). At the same time, there have been offered considerable evidence to support the view that the inhibiting activity of 2, 4–D and related compounds on the growth of the causal fungus is primarily caused by these antibiotics. Since these results were published, the investigation touching this subject has been continued, and it was found that the phenomenon in question is invited as well by 2–methylphenoxyacetic acid (MPA) and 2–chlorophenoxyacetic acid (CPA) both of which too are chemically related to three chemicals above stated. Again, it was also clarified that such nature of inciting antimicrobial substance production depends upon the side chain ($-OCH_2COOH$) of these chemicals. Recently, furthermore, similar studies were extended to other four fungi than *G. olivarum*, and we could confirm that at least the 2 species of them, that is *Gloeosporium kaki* and *Schizophyllum commune*, also act in a similar manner respectively when they were grown in the presence of 2, 4–D. The present paper mainly gives an outline of these results of the writers' experimental studies already published in Japanese (1, 11). Because of the variety of experiments in this work, the details of experimental materials and methods were mentioned in connection with the individual experiments.

Experimental

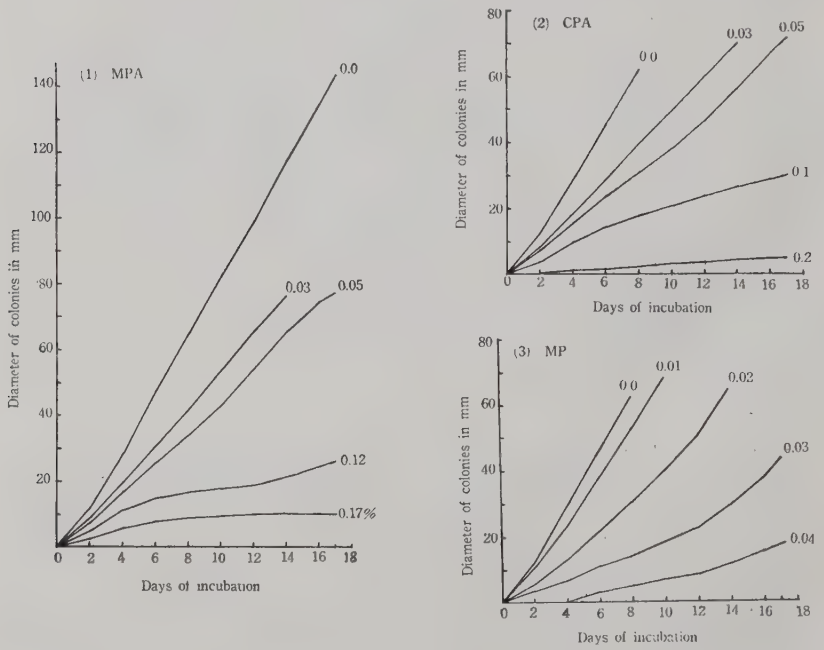
I. Fungitoxic substance production by *Gloeosporium olivarum* grown on media containing either 2–chlorophenoxyacetic acid or 2–methylphenoxyacetic acid.

(1) Growth curves of *G. olivarum* on agar media containing different chemicals.

A motive which led the writer *et al.* to obtain an information that *G. olivarum* is induced by 2, 4–D, 2, 4, 5–T, and MCP to produce certain antibiotic substances responsible for the inhibitory activity of these chemicals

on the growth of the microorganism, was in the fact (2, 6, 8, 9) that the growth curves of the pathogen on media containing these chemicals had been of the "staling" type as compared to being of the "non-staling" type for other chemicals of which chemical structures do not resemble to 2, 4–D. Therefore, with the object of gaining a rough knowledge as to the possibility that the phenomenon of inciting antimicrobial substance production for the present fungus is caused as well by MPA and CPA both of which bear structural resemblance to 2, 4–D, the growth curves of the pathogen were studied with agar media containing these chemicals. At the same time three phenolic compounds, namely 2–chlorophenol (CP), 2–methylphenol (MP), and 2, 4–dichlorophenol (DCP), were also provided for use for the purpose of comparison.

Each of five chemicals chosen was added respectively to peptone-salts agar* having an initial pH of 5.4 to give various concentrations, and the pathogen was cultured on them at 25°C for 17 days using 5–7 Petri dishes per each lot. The diameter of the respective colonies was measured each day. Acetone was used as a solvent for the chemicals under test, so a reasonable question occurred here whether this solvent would affect growth. Hence, media supplied with acetone alone so as to be just the same as the highest level in the lots (0.2 %) were also prepared as second



* Peptone 20 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.4 g, sucrose 50 g, agar 20 g, dist. water 1 *l*.

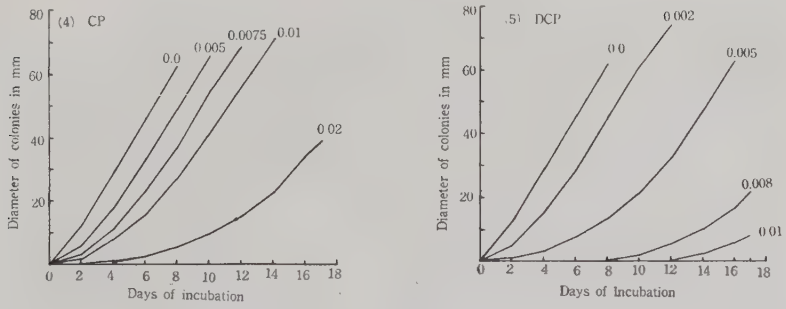


Fig. 1. (1)–(5). Growth curves of *Gloeosporium olivarum* on peptone-salts agar media containing different concentrations (%) of 5 chemicals during 17 day's incubation at 25°C.

The control in (1) alone has been plotted on the basis of the data of the glass tubes in previous papers (2, 6).

control. Since, however, little difference in growth was found between the acetone control and the principal control, the data of acetone control were omitted from the present paper.

Fig. 1 showing mean value of duplicated experiments indicate that the hyphal development is gradually inhibited as the concentration is increased. However, it should be noted here that the growth curve for the MPA and CPA series is of "logarithmic" type (convex upwards) whereas the mycelial curve for other chemicals is of "exponential" type (concave upwards). In other words, the growth rates in the MPA and CPA series decrease with the culture duration, while in the other chemicals the tendency is quite reversed. On the other hand, in all tests not only pH value remained almost constant throughout incubation but also carbon and nitrogen sources had remained in a great deal even at the end of culture. For these reasons, it seemed reasonable to assume that the growth curves for the MPA and CPA series are due to the accumulation of certain staling substances produced during culture in the presence of these chemicals.

(2) Isolation of a crude staling substance from the culture filtrates of *G. olivarum* grown on media containing either 2-chlorophenoxyacetic acid or 2-methylphenoxyacetic acid.

Preliminary research above mentioned has suggested that MPA and CPA also would be useful as incitants of antifungal substance production like 2, 4-D, 2, 4, 5-T, and MCP. In order to obtain more accurate information on the relation, the present experiment was undertaken. In the first place, *G. olivarum* was incubated in quiet culture at 25°C for 12 to 24 days in peptone-salts solutions supplied with 0.1, 0.12 % MPA or 0.1 % CPA, 50 cc of which solution being poured into each Erlenmeyer flask of 200 cc capacity (1st culture). Four flasks were taken at random per each lot, filtered to remove the fungus, and the resulting filtrates were fractionated in order with a variety of solvents according to the procedure presented in Fig. 3.

Fraction II and Fraction III thus obtained were then incorporated respectively into the normal agar media of which quantity was quite identical to the initial solution (200 cc). The causal fungus was again grown on these media (2nd culture), and the diameter of colonies was determined after incubation for 7 days at 25°C. Furthermore, lots devoid of the 1st culture, thus not containing any fraction, were also prepared as control of the 2nd culture. In this manner, the fungitoxicity of both fractions was analyzed. The reason that these fractions were especially selected is based on the fact that the antibiotics produced by addition of 2, 4–D, 2, 4, 5–T, and MCP are all located in fraction III (4, 6, 8, 9) and also that CPA and MPA initially added to media move into fraction II. The mean value of five Petri dishes per each lot is recorded in Table 1. The data indicate that fraction III, a yellowish oil, develops a marked inhibition in either case of both chemicals, excepting 0.1 % MPA. Although fraction II also affected the growth slightly, the resulting effect would probably be due to CPA and MPA respectively which moved to this fraction. The fungitoxicity of fraction III of 12-day cultures with the initial supply of 0.015, 0.02 % CP, 0.02, 0.03, 0.04 % MP or 0.003, 0.005 % DCP was also studied in a manner similar to that described above, but no toxicity could be detected in each of them.

Table 1. Diameter in mm of colonies of *G. olivarum* on agar media containing fractions of the culture filtrates of the causal fungus which was incubated for different periods on liquid media supplied with CPA or MPA

Lot	1st culture		2nd culture	
	Culture duration (day)	Fraction	Diameter of colonies (mm)	Growth index against control
CPA-control*			19.1	30
0.1% CPA	24	II	60.3	94
	12	III	18.9	30
	24	III	12.3	19
MPA-control*			14.7	23
0.12% MPA	12	II	43.7	68
	24	II	59.7	93
	12	III	22.3	35
	24	III	10.5	16
0.1% MPA	12	III	64.0	98
Control**			64.3	100

* 1st culture is absent; 2nd culture was grown on media supplied with either 0.1% CPA or 0.12% MPA alone.

** 1st culture is absent; 2nd culture was grown on normal media without chemicals.

II. Antifungal substance production by *Gloeosporium kaki* and *Schizophyllum commune* grown on media containing 2,4–D.

From the results of the above stated as well as previous studies (4, 5, 6, 7, 8, 9, 10), it is fairly clear that *G. olivarum* produces a certain antifungal

substance respectively when it was grown in the presence of 2, 4-D and related phenoxy compounds.

Therefore, it seemed desirable to ascertain whether such phenomenon is observed in other fungi than *G. olivarum* too or not. Along this line, 2, 4-D culture was conducted with the following 4 fungi as the test organisms: *Gloeosporium kaki* Ito, *Schizophyllum commune* Fr., *Helminthosporium sigmoideum* Cav., and *Cochliobolus miyabeanus* Drechsl.

(1) Growth curves of four fungi on agar media containing 2, 4-D.

In view of the fact that the growth curve of *G. olivarum* on media containing 2, 4-D and related phenoxy compounds all of which induce antimicrobial substance production for the pathogen is of the "staling" type, a preliminary examination was conducted to know the growth curves of four fungi under test on agar media* supplied with different concentra-

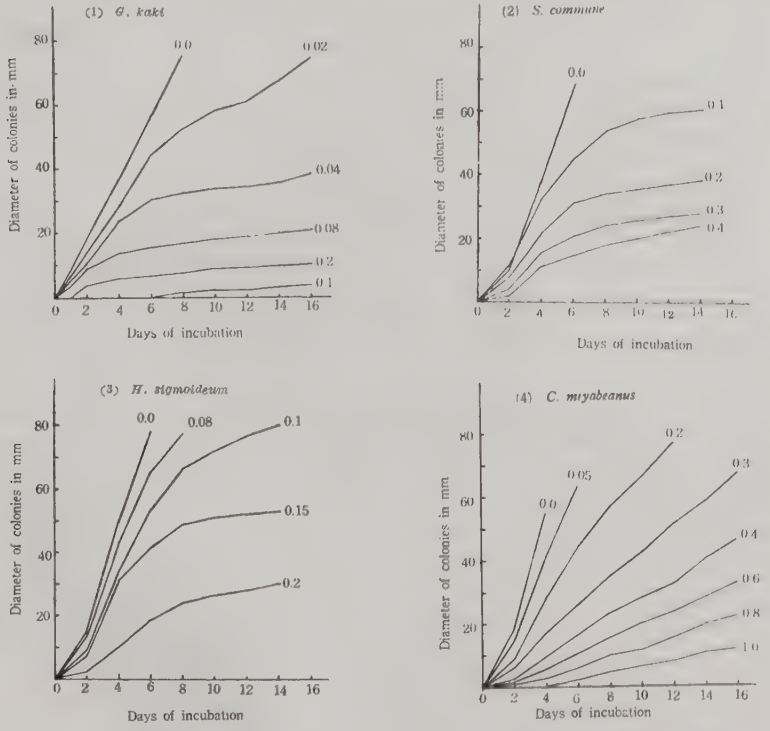


Fig. 2. Grow thcurves of 4 fungi on peptone-salts agar media containing different concentrations (%) of 2, 4-D during 14–16 day's incubation at 25°C.

* In *S. commune* and *H. sigmoideum* 10 g of peptone was added; other components of media are quite similar to those of former footnotes.

tions of 2, 4-D. Other details are similar to the experiment described in (1) of section 1. The results are shown in Fig. 2. It is here seen that 2, 4-D restricts the growth of all the fungi at any given level, progressive inhibition being appeared with increase of concentration. However, the growth curves for *G. kaki*, *S. commune*, and *H. sigmoideum* series were of the "logarithmic" type (convex upwards), indicating a progressive decrease in the growth rate of development, whereas the mycelial curve of *C. miyabeanus* was linear, resembling that of the control cultures without 2, 4-D. In this experiment too, on the other hand, pH of culture media remained almost constant throughout incubation, and carbon as well as nitrogen sources had remained in a great deal even at the end of culture. Richards (12) also has concluded that the average growth rates of *S. commune* on media containing 2, 4-D and 2, 4, 5-T were much lower for the fourth to eighth days than the rates for the first four days.

(2) Isolation of crude staling substances from the culture filtrates of *G. kaki* and *S. commune* grown on media containing 2, 4-D.

Preliminary research now stated has suggested that at leat *G. kaki*, *S. commune*, and *H. sigmoideum* may be induced to produce staling substances by 2, 4-D, as in the case of *G. olivarum*. Accordingly, some efforts were made to isolate the suspected substances in partially purified form.

In the first place, 4 test-organisms were maintained respectively in quiet for 12 days at 25°C in Erlenmeyer flasks of 200 cc capacity, each of which containing 50 cc of peptone-salts solution with an initial supply of 2, 4-D. The concentration of the chemical added to media was 0.04 % for *G. kaki*, 0.1, 0.2 % for *S. commune*, 0.1, 0.15 % for *H. sigmoideum*, and 0.2 % for *C. miyabeanus* respectively. Withdrawing 4 flasks at random per each lot, the resulting culture filtrates were fractionated in order according to

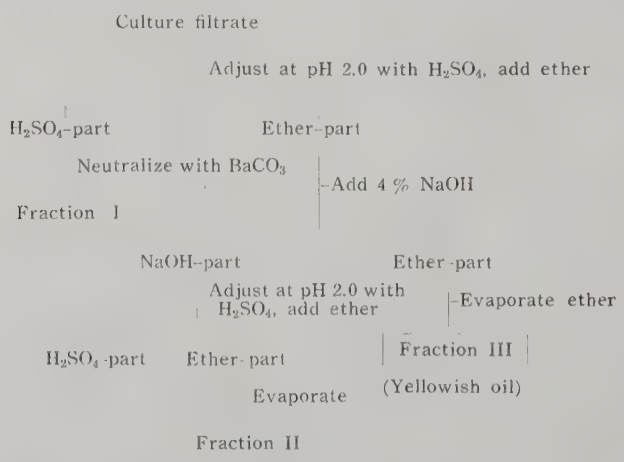


Fig. 3. The fractionation process of culture filtrates.

the procedure presented in Fig. 3. Then fraction I, II, and III thus obtained were added to normal agar media of which quantity was quite identical with the initial solution (200 cc). The 2nd culture of the respective fungi was conducted at 25°C for 6–7 days on these media. The mean value of 7 Petri dishes per each lot is presented in Table 2. The data indicate that fraction III, a yellowish oil, of *G. kaki* and *S. commune* develops a marked inhibition respectively, whereas no inhibition is observed in the same fraction of other two fungi. Fraction I in which the residue of nutrients moves failed to show any inhibition at all in all of the test pathogen.

Table 2. Diameter in mm of colonies of four fungi on agar media containing fractions of the culture filtrates of the respective organisms which were incubated for 12 days at 25°C on liquid media supplied with 2,4-D*

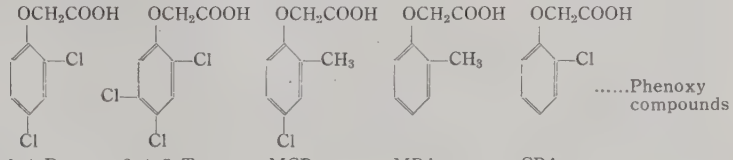
Fungus	Concn. of 2,4-D in 1st culture (%)	Fraction			Control
		I	II	III	
G. kaki	0.04	50.5	33.5	29.7	61.2
S. commune	0.1	67.8	29.6	27.6	86.1
	0.2	70.4	4.1	25.1	86.1
H. sigmoideum	0.1	65.2	12.4	79.9	80.4
	0.15	59.6	±	78.9	80.4
C. miyabeanus	0.2	65.2	23.6	61.1	68.8

* The incubation period in *G. kaki* and *S. commune* is 6 days, and the one in other fungi is 7 days.

Discussion and Conclusion

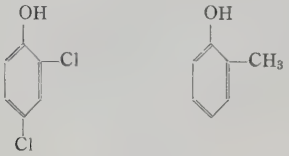
Previously the writer *et al.* (8, 9) expressed the view that the property of inciting antimicrobial substance production for *G. olivarum* is perhaps characteristic of 2, 4-D and related phenoxy compounds, instead of a general phenomenon of the plant growth-regulators. The result of the present paper showing that MPA and CPA also are able to produce the phenomenon must be said to furnish an additional evidence in support of this assumption. Since, moreover, such nature was not detected in MP, CP, and DCP, phenolic compounds of the corresponding phenoxy compounds, it seems reasonable to consider that the nature is based upon the side chain (—OCH_2COOH) of the phenoxy compounds concerned. From the standpoint of ability to cause antimicrobial substance production for *G. olivarum,* the results of the present and previous studies are summarized as follows:

A. Chemicals known to possess such ability.



2, 4-D 2, 4, 5-T MCP MPA CPA

B. Chemicals known to be devoid of such ability or assumed thus in view of the "non-staling" growth-curves of the causal fungus on media containing them.



DCP MP CP .....Phenolic compounds

Potassium α-naphthaleneacetate (NAK) .....Plant growth-regulator
Isopropyl-*n*-(3-chlorophenyl) carbamate (IPC)
Pentachlorophenol (PCP) } .....Herbicides
Uspulun, $CuSO_4$ .....Fungicides

The growth curves of *G. kaki* and *S. commune* on agar media supplied with 2, 4-D was found to be of "staling" type. At the same time, f om faction III of the resulting culture filtrates certain staling substances capable of inhibiting their own growth were isolated respectively. These results are in agreement with the case of *G. olivarum* (2, 4, 6). On the contrary, no fungitoxicity was found in the same fraction of *C. miyabeanus*, in which the growth curve on agar media containing 2, 4-D had been of the "non-staling" type. Considering these things together, it is probable that 2, 4-D does not incite antifungal substance production for this fungus. In *H. sigmoideum* too, fraction III did not exhibit an inhibitory activity against its own growth. However, the growth curves of this fungus on agar media containing 2, 4-D is of "staling" type. In addition, fraction II, in which 2, 4-D initially added to media transfers, develops a marked inhibition in this instance. Therefore it still remains in question as to whether 2, 4-D is able to introduce antifungal substance production for the fungus or not.

In previous papers (5, 7, 9, 10), the author *et al.* concluded that antifungal substances purely isolated from 2, 4-D or MCP cultures of *G. olivarum* play a principal role in the inhibitory activity of these chemicals *in vitro* on the causal fungus, judging from the yield and fungitoxicity, etc. of these antifungal substances. All attempts to isolate the effective agent in purified form from the crude preparation obtained by 2, 4-D cultures of *G. kaki* and *S. commune* have as yet been unsuccessful. Accordingly, it is obscure still whether the agent in the crude material is a single component or not. Since, however, the crude substances reveal an inhibitory activity even when diluted to the concentration about similar to that in the original culture filtrates, it is probable that 2, 4-D gives an inhibiting effect on both fungi by these crude substances respectively rather than by its direct activity on the organisms.

Fungitoxic substances purely isolated from the cultures of *G. olivarum* supplied with 2, 4-D (5, 7), MCP (9, 10), or 2, 4, 5-T (unpublished) have been found to be different each other. So it is expected that a large number of fungitoxic substances would be obtainable by the cultures conducted with

the combination of various phenoxy compounds and fungi.

According to earlier studies (3), in which a great number of fungi was classified to 4 groups on the basis of the resistance to 2, 4-D, *C. miyabeanus* belongs to the most resistant group. Therefore the result of the present paper showing that the fungus in question fails to induce antifungal substance production even when cultured in the presence of 2, 4-D suggests that a part of the different degrees of resistance of fungi to 2, 4-D may be explainable from the degrees of the ability of fungi to incite antifungal substance production in 2, 4-D culture.

Summary

1. When *Gloeosporium olivarum* was grown on agar media containing different concentrations of 2-chlorophenoxyacetic acid (CPA), 2-methylphenoxyacetic acid (MPA), 2-methylphenol (MP), 2-chlorophenol (CP), and 2, 4-dichlorophenol (DCP), the growth curve of the fungus in the series of the former two chemicals was of the "logarithmic" type (convex upwards), indicating a progressive decrease in the rate of development. On the other hand, the mycelial curve in other chemicals was of the "exponential" type (concave upwards), indicating a progressive increase in the rate of development.

From the culture filtrates of the fungus which had been grown in liquid media containing CPA or MPA, there have been isolated crudely a certain fungitoxic substance respectively, which is considered to play a principal role in the inhibitory activity of each chemical on the growth of the microorganism.

2. When *Gloeosporium kaki, Schizophyllum commune, Helminthosporium sigmoideum,* and *Cochliobolus miyabeanus* were grown on agar media containing different concentrations of sodium 2, 4-dichlorophenoxyacetate (2, 4-D), the growth curve of the former three species was of the "logarithmic" type, while mycelial curve of *C. miyabeanus* was linear.

A certain fungitoxic substance of yellowish oil was crudely isolated from the culture filtrates of *G. kaki* and *S. commune* which had been grown in liquid media containing 2, 4-D, but none of that was obtained from normal culture without the chemical. On the other hand, *C. miyabeanus* does not produce such fungitoxic substance even if cultured in the presence of 2, 4-D. The relation in *H. sigmoideum* is not clear as yet. The inhibitory activity of 2, 4-D *in vitro* on *G. kaki* and *S. commune* is considered to be attributable primarily to the fungitoxic substances above stated rather than to the direct effect of 2, 4-D on the pathogen.

3. Nature of inciting a fungitoxic substance production, characteristic of 2, 4-D and related phenoxy compounds, is presumed to be due to the side chain ($-OCH_2COOH$) of these chemicals.

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Studies on the Mucous Layer of the Yeast Cell. I. On its Functions Relating to the Aerial Life.

By

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Xerophytic moulds, which are livable on the surface of dry materials such as glass etc. absorbing moisture from the air, have been studied by Ohtsuki (1943, '50, '51, '53) and Fukuda et al. (1953). Yeasts can also be isolated frequently from dry materials such as leaves and trunks of trees etc. exposed in the air. Shehata, Mrak and Phaff (1955) isolated several yeasts from the interior parts of the body of *Drosophila* and their habitat trees as well, and they found that yeasts isolated seemed to be confined of three genera such as *Saccharomyces*, *Hansenula* and *Pichia*, and asporogenous yeasts. Shifrine and Phaff (1956) obtained similar results on yeasts isolated from bark beetles. Yoneyama (1951) isolated asporogenous yeasts more frequently than sporogenous yeasts from leaves of trees and found some specific relations between the strains and host trees. But it is not clear yet how they are living there.

The present author (1955) found that several strains of yeast were able to live in the solutions excluding both carbon and nitrogen sources. From results of his further studies he came to the conclusion that the vapours of volatile substances as nourishments required for by the yeast might be brought into the media from the air.

Zeller (1927) supposed that the surface of yeast cells would become temporarily mucous correspoding the change of permeability during fermentation. The present author (1950) pursued on this regard by the method of microscopic observation, and did not ascertain the assumption. He stated however that there was a mucous layer with similar components to those of cell walls reported by Salkowski (1921) and Northcote (1952) but other than the yeast gum of Salkowski (1891) and Ohshima (1902).

In this paper will be report a series of experiments dealing with the function of the mucous layer such as absorption of moisture and volatile nourishments from the air to enable yeasts to live on lean habitats.

Methods

Following 12 genera including 24 species of yeasts were used in this study.

Sporogenous species:

Schizosaccharomyces formosensis, Schizosaccharomyces Sautawensis, Schi-

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zosaccharomyces Pombe, Saccharomyces anamensis, Saccharomyces cerevisiae, Saccharomyces Rasse XII, Saccharomyces Rasse II, Zygosaccharomyces mandshuricus, Zygosaccharomyces Sake, Schwaniomyces occidentalis, Pichia polymorpha, Debaryomyces Fabrii, Hansenula anomala Hansen, Hansenula anomala (Kinsi 1-65), Hansenula javanica, Hansenula Saturnus, Hanseniaspora valbyensis, Saccharomycodes Ludwigii.

Asporogenous species:

Mycoderma Chevalieri, Mycoderma vini, Pseudosaccharomyces apiculatus, Torula minuta, Torula rubescens, Torula utilis.

In order to isolate the mucous layer, synthetic liquid medium containing 0.1% $MgSO_4$, 0.2% KH_2PO_4, 0.2% $(NH_4)_2SO_4$ and 10% sucrose in an Erlenmyer flask (300 cc) was sterilized, inoculated and cultured for twenty days at room temperature.

To find out nutriment sources available for yeasts in dry places, following two kinds of media were prepared. For the purpose of seeking carbon sources a kind of medium containing 0.1% $MgSO_4$, 0.2% KH_2PO_4 and 0.2% $(NH_4)_2SO_4$ was used, and in like manner to search for the nitrogen sources another medium containing 0.1% $MgSO_4$, 0.2% KH_2PO_4 and ethylalcohol was used. Others will be mentioned in the corresponding experiments.

The inoculation was controlled to equalize the initial concentration of cells with the standard gradation number (0). After the incubation time of about twenty or thirty days, the growth of the yeast was measured mainly by comparing the degree of turbidity of a medium with the standard which was prepared as follows. One loopful organism was taken out of a colony on a solid medium, suspended into 10 cc of water (5) and diluted gradually into 1/5(4.5), 1/10(4), 1/50(3.5), 1/100(3), 1/500(2.5), 1/1000(2), 1/5000 (1.5), 1/10000(1), 1/50000(0.5) and 1/100000(0) with water. The figures enclosed in the parentheses were used to designate gradation of turbidity. Observed values of turbidity less than (1) were estimated as the symptom of negative.

The humidity of the aerial culture was controlled with $CaCl_2$ solution at 20°C as follows.*

$CaCl_2$ (mol)	0.0	0.25	0.50	0.75	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Rel. humidity (%)	100	98.0	96.6	95.2	93.4	89.7	85.4	80.0	73.9	68.5	61.5	59.3	47.0

Closed glass chambers in which test tubes with media were set were partly filled with $CaCl_2$ solutions of various concentrations.

Isolation of mucous substance from yeast colonies was carried out by the following procedure mentioned in the former report (Kaibara, 1954). The mucous substance was separated by strong shaking of yeast cells in

*Relative humidity responding to molecular concentration of anhydrous $CaCl_2$ (estimated by Fukuda in 1953).

water, filtrated and finally precipitated by adding methanol. The precipitate was dissolved in water, dialysed in running water for three days, and the mucous substance precipitated with methanol repeatedly was prepared as greyish horny substance.

Experiments and Results

Experiment 1. **Nutritive values of gaseous carbon compounds.**

Effectiveness of the vapours of carbon compounds on growth was tested in the media without carbon sources.

CO, CO_2, CH_4 and coal gas were introduced through a duct from each gas generator into the chambers in which media in test tubes were put. The liquid substrates were dropped by glass capillary into the chambers keeping from mixing directly with the media.

The results are shown in Table 1. Among the applied substances methylalcohol, ethylalcohol and esters were available as the carbon nutri-

Table 1. Growth in liquid media in the presence of vapour of various carbon compounds.

Substrates / Strains	Acetic acid	Methyl-alcohol	Ethyl-alcohol	Acetic-methyl ester	Acetic-ethyl ester	Acetic-buthyl ester	Acetic-amyl ester
Saccharomyces anamensis	1	1	3.5	1	1	1	1.5
Pichia polymorpha	1.5	1	5	1	3.5	1	1.5
Debaryomyces Fabrii	2.5	1	4	0	1	1	2
Hansenula anomala H.	3.5	1	4	1	4	1	1
H. anomala (Kinsi)	4	1	3.5	1	3	1	1
H. javanica	3	1	4	1	4	1	1
H. Saturnus	3	1	3	1	3.5	1	1
Mycoderma Chevalieri	4.5	0	2	1	1	0	1
Torula minuta	0	1	2.5	1	1·5	1	1
T. rubescens	0	0	3.5	1	3.5	1	1.5
T. utilis	0	0	4	1	1.5	1	1

ment to several strains such as *Saccharomyces anamensis, Pichia polymorpha, Debaryomyces Fabrii, four species of Hansenula, Mycoderma Chevalieri* and three species of *Torula.*

Ethylalcohol was most favorable, acetic-ethylester, methyl-alcohol and the other esters were unfavorable. Acetic acid was utilized rather well by the majority of the strains mentioned above, but not by Torula.

Experiment 2. **Nutritive value of nitrogen salts and vapours.**

0.2% of several salts (ammonium salts, nitrates, nitrite and urea) were previously added to the media before inoculation, and the other volatile nitrogen and carbon sources were added sparingly after the inoculation.

The results are shown in Table 2. Ammonium salts, nitrates and urea were almost equally effective for the growth of all strains, but nitrite was

Table 2. Growth in liquid media in the presence of various nitrogen salts or vapours.

Substrates / Strains	Salts (0.2%)							Vapours (very low concentration)		
	$(NH_4)_2SO_4$	$(NH_4)_2HPO_4$	$(NH_4)NO_3$	$NaNO_3$	KNO_3	$CO(NH_2)_2$	$NaNO_2$	Ammonia	Skatol	Indol
Saccharomyces anamensis	4.5	4	4	4.5	4.5	3.5	1	3.5	<1	<1
Pichia polymorpha	4.5	4	4.5	4.5	4	3	1	3.5	2	1
Debaryomyces Fabrii	4.5	4	4.5	4.5	4.5	4	1	2	3	<1
Hansenula anomala Hansen	4	3.5	3.5	4	4	3.5	<1	2	2	1.5
H. anomala (Kinsi)	4	4	3.5	4	3.5	3	<1	2	1.5	1
H. javanica	4.5	4	3.5	3.5	4	3.5	<1	1.5	1	<1
H. Saturnus	4	3.5	4	4	4	3.5	<1	1.5	1.5	<1
Mycoderma Chevalieri	3.5	3	3	3	2.5	3	<1	3	1	<1
Torula minuta	3	2.5	2.5	2.5	3	2.5	<1	2	1.5	1
T. rubescens	3.5	2.5	3	3	3	2.5	<1	2	2	1
T. utilis	4.5	3.5	4.5	4	4.5	3	<1	3	1.5	2

unfavorable. Among the volatile substances, the vapours of ammonia, skatol and indol were available when their concentrations were very low, while they were harmful in high concentration: ammonia was best and indol was worst.

Experiment 3. **Nutritive value of vapours of volatile organic matters.**

Three series of basal media were prepared as follows.

1. 0.1% $MgSO_4$+0.2% KH_2PO_4 (without carbon and nitrogen sources).
2. ,, + ,, +0.2%$(NH_4)_2SO_4$ (without carbon sources).
3. ,, + ,, +ethylalcohol (vapour) (without nitrogen sources).

Vapour of volatile organic substance was supplied to the media in chambers.

The results are summarized in Table 3. Among the 24 species tested, the strains which utilized the vapours of carbon and nitrogen compounds in the experiments 1 and 2, were able to grow, while the others did not.

The addition of excrements of mankind, dogs and domestic fowls, Miso, soy and Koji produced good effect on the growth in the media of all the series.

Sewer soil, vegetable manure, mushrooms and rotten entrails of fishes and squids permitted growth only in the medium 3, which seems to indicate that vapours of these matters would supplied only nitrogen nutriment.

Many organic substances permitted the organisms to grow only in the medium 2. Among them manufactured foods (cheese, lees of Sake and bread) and fruits as oranges, apples, peaches and lemons were most favorable; crude vegetable oils of lemons, camellias, sesames, cottons and olives, twigs of cherries, peaches, figs, pears, grapes and plums and plum flowers were utilized to a small degree; while the vapours of salted radish, leaves of pines, cryptomerias, japanese crypresses, laurels and xanthoxylums, and flowers and leaves of freesias, daphnes, wall flowers, chrysanthemums, gardenias and clerodendrons were not utilized at all.

Experiment 4. **Nutritive value of vapours in the air.**

The basal media mentioned in the experiment 3 were used, but ethylalcohol was added to one series in liquid condition.

After inoculation the preparations were brought to various places such as work shops (breweries of Miso, Soy and Sake, and bakeries); stores of flowers, books, fruits, provisions and candies; school rooms; platforms of stations; roofs of buildings; depositaries of vegetable manure heap and toilets. They were kept there for about thirty days.

Among the places tested, the growth of all the cultures was negative in the school rooms, the book stores, on the roofs of buildings and the platforms of stations. The results on the other places were positive though some were favorable and others were less favorable as shown in Table 4. Only the strains grown positively in the experiments 1, 2 and 3 grew in this experiment also.

1. The growth was positive in all basal media in the breweries of Miso and Soy. Therefore, it seemed that there were carbon and nitrogen nutritive vapours in the air.

2. The growth was favorable in the basal medium 2 in the breweries of Sake, the bakeries, the fruit stores and the provision stores; and in

Table 3. Growth in liquid media in the presence of vapours of various organic compounds.

Series of medium in which growth was possitive.	Series 1, 2 and 3							Series 2																						Series 3		
Substrate	Excrements			foods			foods	Fruits							Vegetable oils				Twigs							Flower							
Strains	Mankind	Dogs	Domestic fouls	Miso	Soy	Koji	Cheese	Bread Lees of Sake	Oranges	Apples	Persimmons	Peaches	Lemons	Lemons	Camellias	Sesames	Cottons	Olives	Cherries	Peaches	Figs	Pears	Grapes	Plums	Plums	Sewer soil	Vegetable manure heap	Mushrooms	Fishes	Squids
Saccharomyces anamensis	4	2	3	1	1	<1	1	4	3.5	3.5	3	2.5	3	1.5	1	1	1	1	1.5	1.5	1	1	1	2	1	<1	1	1.5	2	2	1
Pichia polymorpha	3.5	2	1	2.5	2.5	1.5	3	4.5	4.5	4	3.5	4	4	3.5	2	1	1.5	1	1.5	1.5	1.5	1	1.5	2.5	2	1	3	2	4	1.5	2
Debaryomyces Fabrii	2	1.5	1.5	1	2	<1	2	3	3.5	4	3	4	4	2.5	<1	1	<1	<1	1	1.5	1	1.5	1	1.5	1	0	<1	<1	4	1	<1
Hansenula anomala Hansen	3	2	2	1	1	<1	2	4	4	2.5	3	3	3	2	1	1	1	1	1	2	<1	1	1	2	1	1.5	<1	<1	3	1	<1
H. anomala (Kinsi)	2	1	1	1.5	2	<1	2	3	4	3	3	2	2.5	1	1	1	1	1	1	1.5	<1	1	1	2	2	1	<1	<1	4	<1	0
H. javanica	1.5	1	1	1	1.5	<1	1.5	4	4	2.5	2.5	3	2.5	1.5	1	1	1	1.5	1	2	1	1	<1	2	<1	1	<1	<1	3	1	1
H. Saturnus	1.5	1	1	1	1	<1	1.5	4	3.5	2	3	2	3	1.5	1	1	1	1.5	1	2	1	1	1	1.5	1	1	<1	<1	3	1	<1
Mycoderma Chevalieri	3.5	2	2	3	2	1	1.5	3.5	3	2	1.5	1.5	1	0	<1	<1	1.5	1.5	0	1	<1	1	<1	1.5	<1	0	1.5	3	3	1	1.5
Torula minuta	1	1	<1	2	2	1.5	2	3.5	4	3	3.5	2	3.5	2	<1	1	0	0	2	0	1.5	1.5	1	1.5	2.5	1.5	2	1.5	4.5	1	1
T. rubescens	1	2	1	1	1	<1	2.5	2.5	3.5	3.5	3.5	3	3.5	3	2	1	2	<1	2	1.5	1.5	1.5	1	2	1.5	1	2	2	3	1	1
T. utilis	3	1.5	1	3	3.5	2	3	4	4.5	4.5	3.5	4	4	3	1.5	<1	1.5	2	1	1	1	1	<1	1.5	1	1.5	2	1.5	3	2	2

Table 4. Growth of yeasts in various places.

Series of medium in which the growth was positive	Series 1, 2 and 3		Series 2							Series 3	
Places / Strains	Breweries		Breweries of Sake	Bakeries	Fruit stores	Provision stores	Flower stores	Candy stores	Depositaries of vegetable manure heap	Toilets	
	Miso	Soy									
Saccharomyces anamensis	2.5	4	2	2.5	3	1.5	<1	<1	1	2	
Pichia polymorpha	3.5	3.5	3	3	3	2	<1	1	1	2	
Debaryomyces Fabrii	3	3	2	2.5	3	1	0	0	1	1	
Hansenula anomala Hansen	2.5	3	2	3.5	2.5	2	<1	0	1	2	
H. anomala (Kinsi)	3	3.5	2.5	3	3	2	0	0	<1	1	
H. javanica	2	2.5	1.5	2	2.5	1.5	0	1	<1	1	
H. Saturnus	2	2	1	1.5	2	1.5	0	<1	<1	1	
Mycoderma Chevalieri	2	2.5	1.5	2	1	1	0	0	2	1.5	
Torula minuta	3	3.5	2.5	3	2.5	1.5	0	1	1	<1	
T. rubescens	3	3	2.5	2	3	2	<1	<1	2	<1	
T. utilis	3.5	3.5	3	2.5	3	2	<1	1	2	2	

the flower stores and the candy stores they grew only to a small degree in the same medium. Therefore, it seemed that there was only carbon nutritive vapour in the air.

3. On the depositaries of vegetable manure heap, the growth was somewhat positive only in the basal medium 3, but not clear in the basal medium 2. Therefore, it seemed that there was only nitrogen nutritive vapour in the air. More-over, in the toilets the growth was limited only in the basal medium 3.

Experiment 5. **Nutritive value of dust extract.**

Dust was gathered with a hair pencil, put into distilled water in the concentration of 1% and stayed for 24 hours. The extract was transfered into test tubes and sterilized.

The results are shown in Table 5. The growth of almost all the strains tested was positive in every extract. The most favorable growth was attained by the strains which utilized the organic vapours as mentioned in

Table 5. Growth of yeasts in dust extract.

Localities of dust / Strains	Roof	Press	Floor	Trunks				Leaves								
				Pinus densiflora	Cryptomeria japonica	Prunus serrulata	Castanea crenata	Pinus densiflora	Sciadopitys verticillata	Sasa Veitchii	Shiia cuspidata	Photinia glabra	Ilex integra	Rhododendron obtusum	Buxus japonica	Eurya japonica
Schizosacch. formosensis	0	0	0	0	0	0	<1	0	1	0	1	1	1	1	1	1
Schizosacch. Sautawensis	0	0	0	0	0	1	<1	1	<1	1	1	1	<1	1	1	1
Schizosacch. Pombe	0	1	0	0	1	0	1	<1	<1	0	<1	<1	<1	1	1	<1
Sacch. anamensis	0	0	<1	2	1	2	1	1	1.5	1.5	1	1.5	2	2	1.5	3
Sacch. cerevisiae	0	0	0	<1	1	1.5	1	1.5	1	<1	1	1	1	1	1	1
Sacch. Rasse XII	0	0	0	2	2	1	1	1.5	1	1	1	1	1.5	1	1	2
Sacch. Rasse II	0	<1	0	1	1	2	2	2	1	1	2	2	1.5	2	2	2
Zygosacch. mandshuricus	0	0	0	1	1	2	2	1	0	1	3	1	<1	2	1	1
Zygosacch. Sake	0	0	1	1	1	2	2	1	1	1	2.5	2	1	2	2	2
Schwaniomyces occidentalis	0	0	<1	1	2	2	2	1	1	2.5	1.5	1	2	1.5	1.5	2
Pichia polymorpha	0	1.5	1	2	3	3	3	3.5	3	3.5	3	3	3.5	3.5	3.5	4
Debaryomyces Fabrii	0	1	1.5	3	3	3	3	3	3	2.5	3	3	3.5	3	2.5	3
Hansenula anomala Hansen	0	1	1.5	2.5	2	3	3	2	2.5	3	2.5	3	3	3.5	3	3
H. anomala (Kinsi)	0	<1	1	1	1	2	2	2	2	2	2	2	2	3	2.5	3
H. javanica	0	0	1	2	1	2	2	2	2	3	3	2	2	3	2.5	2.5
H. Saturnus	0	1	<1	1	1	1	2	1.5	2	3	2.5	1	2	3.5	2.5	3
Hanseniaspora valbyensis	0	<1	<1	1	1	2	1	<1	<1	0	2	1	2	1	1.5	2
Saccharomycodes Ludwigii	0	<1	<1	1	<1	1	1	<1	0	0	<1	<1	0	1	0	1
Mycoderma Chevalieri	<1	1	<1	1.5	1.5	2	2	2	2.5	1.5	2.5	2	2.5	2	2	3
M. vini	0	1	<1	1	1	1	2	1	<1	<1	1.5	0	3	2	<1	2
Pseudosacch. apiculatus	0	0	0	0	0	1	<1	0	0	0	1	1	0	0	<1	1
Toru a minuta	<1	1	1	2	1.5	2.5	2.5	3	3	2	3	3	3.5	3	3.5	3.5
T. rubescens	<1	2	1.5	2	2	2.5	2	3	3	3.5	3	3	4	3.5	3.5	4
T. utilis	<1	1	1	3	2	3	3	3	3	3.5	3	3	3.5	3	3	4

the experiments 1, 2, 3 and 4. It was observed that the dust on the leaves and trunks of trees was generally more valuable than that on the roof, the press and the floor. Consequently the surfaces of leaves and trunks may be good habitat for yeasts.

Experiment 6. **Nutritive value of the mucous substance.**

To ascertain if the mucous layer is available for carbon nutriment of yeasts, the isolated mucous substances of several strains such as *Saccharomyces anamensis*, *Pichia polymorpha*, *Debaryomyces Fabrii*, *Hansenula anomala H.*, *Mycodema Chevalieri*, *Torula minuta*, *Torula rubescens* and *Torula utilis* were prepared. The four series of media were prepared as follows.

1. 0.1%$MgSO_4$+0.2%KH_2PO_4+0.2%$(NH_4)_2SO_4$.
2. ,, + ,, + ,, +5% mucous substance.
3. ,, + ,, + ,, +1% ethylalcohol.
4. ,, + ,, + ,, +5% mucous substance +1% ethylalcohol.

The strains from which mucous substances were prepared were inoculated in the media. After the incubation for thirty days, the dry weight of mycelia was measured.

The growth occured in the medium containing ethylalcohol only or both ethylalcohol and the mucous substance, but there was no difference of the dry weight between the two. No growth was seen in the medium containing the mucous substance as the sole source of carbon compound. Therefore, it is concluded that the growth in the medium 4 is due to the addition of alcohol, and the mucous substance does not play the role of carbon source at all.

Experiment 7. **Yield of the mucous substance.**

The mucous layer of all the test strains except *Saccharomyces* were isolated to compare the yield. The results obtained are shown in Table 6. In the table the results of electron microscopic observation are noted and compared with the yield.

Although the yields were variable considerably according to strains, the general trend was that the yield of asporogenous strains tested was higher than the sporogenous strains.

More-over, it was recognized in every strain that there was close relationship between the yield and the quantitative feature observed by electron microscope as the table shows. This fact means that the isolated substance was identical with the mucous layer.

Experiment 8. **Solubility of the mucous substance.**

Preparations were made of the products of the strains which could utilize the organic vapours. The results are summarized as follows.

1. The preparations were dissolved easily in dilute alkali solution,

Table 6. Yield of the mucous substance and the features observed by electron microscope.

Strains	Yield of the mucous substance per dry-weight 1g of mycelia (mg)	Election microscopic observation of the mucous layer.
Schizosacch. formosensis	17.5	M.L. adhered to the cell and spread on C. M. a little.
Schizosacch. Sautawensis	20.1	Do
Schizosacch. Pombe	7.2	M.L. was found little around the cell and on C.M.
Zygosacch. mandshuricus	12.3	M.L. adhered little to the cell, and spread a little on C.M.
Zygosacch. Sake	20.5	Do
Schwaniomyces occidentalis	23.2	Do
Pichia polymorpha	6.9	M.L. was found little. The cell was naked.
Debaryomyces Fabrii	10.1	Do
Hansenula anomala Hansen	5.2	Do
H. anomala (Kinsi)	6.7	Do
H. javanica	10.0	Do
H. Saturnus	15.0	Do
Hanseniaspora valbyensis	13.0	Do
Saccharomycodes Ludwigii	8.0	Do
Mycoderma Chevalieri	55.3	M.L. was found abundantly around the cell and on C.M.
M. vini	40.0	Do
Pseudosacch. apiculatus	30.0	Do
Torula minuta	70.2	Do
T. rubescens	126.0	Do
T. utilis	36.0	Do

Note: M.L. = Mucous layer. C.M. = Collodium membrane.

precipitated by Fehling's solution and could not be salted out by saturated $(NH_4)_2SO_4$ solution. Therefore, it may be concluded that the preparations are similar to yeast gum, but not to glycogen.

2. It dissolved in water with some difficulty. They swelled gradually in it and became finally colloidal by strong shaking or stirring. This feature distinguished the substance from the yearst gum, for the latter dissolved easily in water.

3. They were wholly insoluble into organic solvents such as methanol, ethanol, ethylether, aceton, chloroform and benzene.

Experiment 9. **Absorption of moisture and ethylalchol gas by the mucous substance.**

To measure the power of absorbing moisture and alcohol gas mucous substances of the five strains (*Sabcharomyces anamensis, Pichia polymorpha, Mycoderma Chevalieri, Torula minuta and Torula rubescens*) were prepared. Dry weight of the preparations was measured and they brought into glass chambers at the bottom of which calcium chloride solutions of various concentrations were placed to control each relative humidity. The preparations were kept in the chambers for three days at 20°C. The degree of moisture absorption was represented in percentages per dry weight of the preparations.

The results were compared with several allied substances such as agar and gelatine. The preparations in question possessed very high power to absorb moisture in the air saturated and nearly saturated with water. Among the substances for comparison yeast gum and cane sugar deliquated by absorbing much moisture, gelatine and agar were somewhat less powerful, and the others much less than the preparation.

The preparations desiccated were brought into a glass chamber containing gas saturated with alcohol for two days at 20°C. For measuring alcohol, distillates of the preparations were titrated quantitatively with potassium permanganate solution. As Table 7 shows, it was found that every prepara-

Table 7. Absorption of moisture or alcohol gas in the saturated atmospheric humidity.
(proportion to dry weight of preparation)

Preparations	Water absorbed (%)	Alcohol gas absorbed (%)
Mucous subtance of *Saccharomyces anamensis*	60.0	29.7
Do *Pichia polymorpha*	57.0	32.4
Do *Mycoderma Chevalieri*	47.0	30.5
Do *Torula minuta*	53.2	32.8
Do *Torula rubescens*	55.0	33.4
Starch paste	23.0	
Filter paper	9.0	
Agar	31.0	
Gelatine	33.4	
Glycogen	14.0	

tion absorbed about 30% of alcohol gas.

The degree of moisture absorption in various humidities are shown in Fig. 1. The preparations in question possessed excellent absorptive power

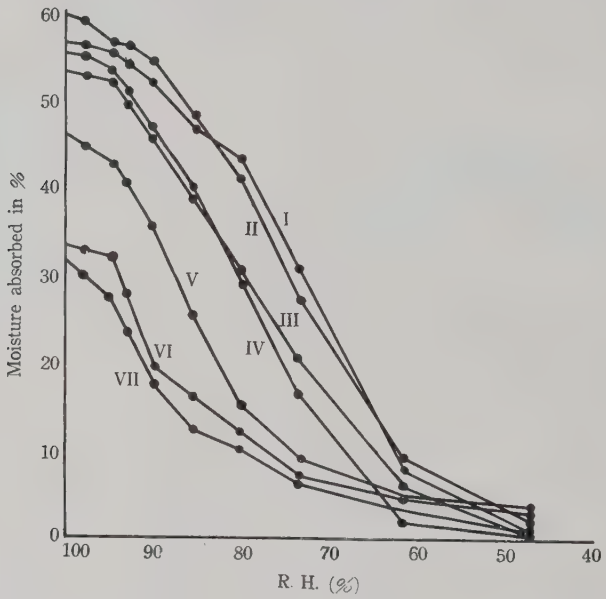

Fig. 1. Moisture content absorbed under various relative humidities.
I. *Pichia polymorpha* II. *Saccharomyces anamensis*
III. *Torula rubescens* IV. *Torula minuta*
V. *Mocoderma Chevalieri* VI. *Gelatine* VII. *Agar*

in comparison with gelatine and agar in the broad range of humidity, but the powers almost equal to the controls in the low humidity. Such powerful moisture absorption of the mucous substance in the broad range of humidity must be favorable to water economy of yeasts in dry condition.

Exeperiment 10. **Viscosity and surface tension of the mucous substance.**

Viscous feature of the mucous layer observed by electron microscope is illustrated in Fig. 2. The 5% mucous substances of the five strains were used to measure the viscosity and the surface tension. Micro Ostwald viscosimeter with 2 cc volume and glass capillary with 0.5 mm diam. were applied to their measurement. Viscosity values of yeast gum, glycogen and gum arabic were also shown in Table 8 for comparison. The relative viscosity of the preparations was above 2.5 at 20°C. This value

Fig. 2. The viscous feature of the mucous layer by electron microscopic observation.
(*Torula rubescens* ×10000)
Y.....Yeast cells
M.....Mucous substance

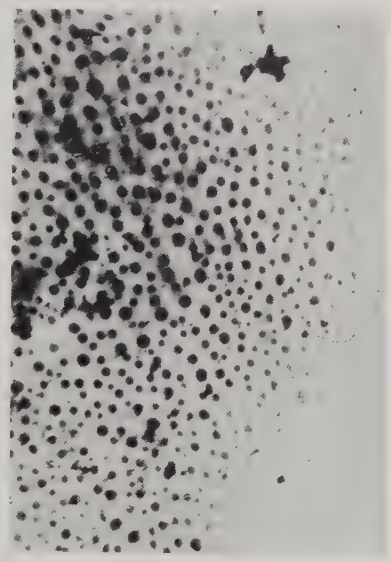

Fig. 3. The mucous substance spread on collodium membrane. Many granules are seen in the medium.
(*Mycoderma Chevalieri* ×10000)

Table 8. Viscosity and surface tension.

Preparations	Relative viscosity (20°C)	Surface tension (20°C)
Mucous substance of *Saccharomyces anamensis*	2·70	98
Do *Pichia polymorpha*	2. 90	97
Do *Mycoderma Chevalieri*	2. 86	98
Do *Torula minuta*	2. 75	97
Do *Torula rubescens*	2. 83	96
Yeast gum	2. 60	98
Glycogen	1. 75	98
Gum arabic	4. 05	96
Water	1. 00	100

was nearly equal to that of yeast gum, higher than glycogen and lower than gum arabic. The values of the surface tension denoted in the same table show approximately near that of water.

Experiment 11. **Adhesion of the mucous substance to dust particles.**

As the preparations of the mucous substance were viscous and possessed excellent power to absorb moisture, it is expected that they adhere strongly to dust particles floating in the air. Indeed, it was frequently observed by electron microscope that contaminated granules of the medium were in the mixed mucous substance as shown in Fig. 3.

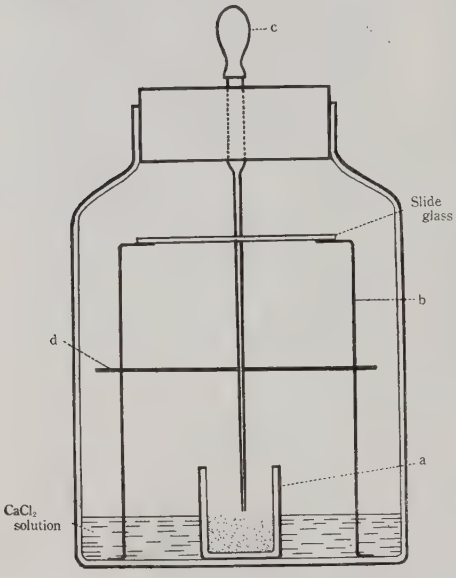

Fig. 4. The apparatus to test the adhesive power of the mucous substance to dust.

To measure the power in question, solutions of the preparations were dropped on slide glasses, desiccated and brought into chambers of constant humidities. In each chamber a little glass tube (Fig. 4. a) containing dust gathered on a roof was placed. The slide glass with mucous substance underside was placed on the supporter (Fig. 4. b) and kept for three days at 20°C. By pressing gum cap (Fig. 4. c), dust particles were spread upwards through the margin of the obstructive plate (Fig. 4. d) placed between the slide glass and the tube.

The adhesive power in various humidties was measured under microscope by taking count of dust particles which adhered to the prepartions. The number of dust particles which adhered to each preparation amounted practically to about twenty per 1 mm^2 in the air of saturated humidity. Table 9 shows the average percentages of dust particles in comparison with the value of saturated humidity.

The preparations possessed about equally high power above humidity 80%, but the power decreased rapidly below it.

Experiment 12. **Aerial culture.**

It was examined if the yeast strains could be cultured in dry conditions, when vapours of volatile substances and dusts were given to them. Five strains were employed. In two series (I and II) of experiments yeasts were inoculated directly on cleaned slide glasses, in second two series (III and IV) of experiments on similar glasses which were smeared with mucous substance of each strain and fifth series (V) on a glass which was smeared with a mixture of 0.1% $MgSO_4$ and 0.2%KH_2PO_4. Each glass was desiccated before inoculation. The slide glasses with yeast suspensions

Table 9. Adhesive power to dust particles in various R.H.

Strains from which mucous substances were prepared \ Humidity (%)	100	98	96.6	95.2	93.4	89.7	85.4	80.0	73.9	68.5	61.5	59.3	47.0
Saccharomyces anamensis	100	98	95	88	89	75	70	70	52	20	15	7	4
Pichia polymorpha	100	92	87	94	90	82	80	82	50	25	17	9	8
Mycoderma Chevalieri	100	96	95	96	82	85	75	70	32	28	30	10	4
Torula minuta	100	100	95	97	91	81	78	80	53	40	35	3	5
Torula rubescens	100	101	99	97	86	84	80	82	60	41	20	12	5

were dried soon after inoculation at room temperature. Finally they were placed in test tubes where the humidity was regulated by calcium chloride solutions. Cultures of four series were supplied with vapours of ethylalcohol and ammonia (II, III and V), or dusts (IV). Cultures of rest one series (I) were left nothing supplied.

They were cultured for fifty days at 20°C. In both series I and II, no colony formation occured. Results of experiments of other three series (III, IV and V) are shown in Table 10. In series III only *P. polymorpha*, *M. Chevalieri* and *T. rubescens* grew, when it was saturated or nearly saturated. The colonies were formed on the part of glass smeared mucous substances, and spread more or less extensively to naked glass surface

Table 10. Colony formation in aerial culture.

Cultural series	Series III M.S. and V. are present				Series IV M.S. and D. are present				Series V I.S. and V. are present			
Humidity (%)	100	98.0	96.6	95.2	100	98.0	96.6	95.2	100	98.0	96.6	95.2
Saccharomyces cerevisiae	−	−	−	−	+	±	−	−	−	−	−	
Zygosaccharomyces mandshuricus	−	−	−	−	+	+	−	−	-		−	−
Pichia polymorpha	+	+	±	−	+	+	−	−	+	+	−	−
Mycoderma Chevalieri	+	+	±	−	+	+	−	−	+	+	−	−
Torula rubescens	+	+	±	−	+	+	−	−	+	+	±	−

Note: M.S. = Mucous substance. I.S. = Inorganic salts. V. = Vapours of ethylalcohol and ammonia. D. = Dust.

especially when it was saturated. The more humidity decreased, the less growth of yeast was, and at about 97% R.H. colonies were formed no more. *Sacch. cerevisiae* and *Zygosacch. mandshuricus* did not form any colony even when it was saturated. In the series IV, all strains tested could grow, and in the series V it was the same as series III.

In the series I complete absence of nutriments and in the series II lack of inorganic salts inhibited growth of yeasts. The results of the series III indicate that yeasts were supplied all necessary nutriments. Therefore, it seems that the mucous substance, perhaps owing to its impurities, was effecties was inorganic nutriment source.

Concerning *Sacch. cerevisiae* and *Zygosacch. mandshuricus*, it is considered that they did not grow in the series II, III, and V owing to inability to utilize the gaseous substances.

Through these results, it is concluded that many strains of yeast possess the ability to grow on dry media taking moisture and nutriment directly from the air, even if they are supplied with no water and nutriment from the media.

Discussion and conclusion

Many volatile substances are contained in the ingredients of liquid media for cultivation of yeasts, but those have never been experimented formerly in the form of their vapours.

In this paper, it was found that yeasts could utilize vapours of organic substances as carbon or nitrogen sources and dust as a perfect nutriment. It was found also that the mucous layers of yeasts are functional for absorbing water vapour and other vapours and for catching dusts in the air. More-over, Dr. Kobayashi suggested that the existence of the layer around the cell wall of yeast was effective in preventing the contact of the yeast cells with each other and in keeping the absorbing surface.* So the layers can accumulate moisture and the nutritious substances by their strong absorbing power when the weather is wet or nourishments are abundant and can protect the yeast life when the weather is dry or nourishments are poor.

The mucous substances isolated were so viscous and their surface tension was so much lower than that of water that this layer must be effective to fix yeast cells on dry media such as leaves and trunks of trees etc.

Asporogenous strains possessed larger amounts of mucous substances than sporogenous strains. It seems that the existence of mucous layers supports their life in dry habitat, resulting in making them tolerate unfavorable conditions even though they lack the ability of spore formation.

From these facts, it is clear that yeasts grow in aerial habitat, receiving moisture as well as nourishments containing carbon, nitrogen and

*The author is greatly indepted to Dr. Yoshio Kobayashi for his valuable suggestion.

minerals from the air, even though substrate on which yeasts live is dry and sterile. However, only a few strains could utilize vapours of organic compounds even though almost all strains utilized dust in the air, so it may be presumable that the strains which can utilize vapours may dominate in aerial habitat.

Yeast strains which are most suitable for aerial life are thought to be asporogenous yeasts belonging to *Torula* etc. and sporogenous yeasts belonging to *Saccharomyces, Hansenula* and *Pichia* etc., which can utilize vapours. This reasoning of the aerial inhabitants explains also why these yeasts are collected more frequently than others on dry media.

Summary

The aerial viability of yearts was experimented by using test strains of 12 genera, 24 species and following results were obtained.

1. Vapou s of alcohols, esters and acetic acid were used as the sole source of carbon nutriment for several strains such as *Saccharomyces anamensis, Pichia polymorpha, Hansenula anomala H.* etc., *Debaryomyces Fabrii. Mycoderma Chevalieri* and *Torula minuta* etc..

2. Vapours of ammonia, skatol and indol in small dosis were utilized for the sole source of nitrogen nutriment.

3. Vapours of various volatile organic matters such as foodstuffs, fruits, twigs, excrements, sewer soil, vegetable manure, mushrooms and rotten entrails of fishes and squids etc. were available either for carbon or nitrogen sources.

4. Vapours in the air at places such as brewe ies, bakeries, fruit stores, provision stores, vegetable manure heaps and toilets etc. were also available likewise.

5. Extract of dust collected on the surface of leaves and t unks of trees etc. was utilized by all the test strains for a perfect nutriment.

6. Mucous substance surrounding yeast cell did not serve as carbon source, but contained mineral nourishments.

7. The mucous substance swelled in water and turned into colloidal solution.

8. The mucous substance possessed strong power to absorb moisture and gaseous substances, and this nature makes it possible for yeasts to get its living in dry and sterile habitat.

9. The mucous substance was viscous, and its surface tension was somewhat lower than that of water. By the properties, it may be possible to adhere to dry substrates.

10. Aerial cultures of several strains were performed under constant atmospheric humidity. They could grow on glass surface even at lower humidity when dusts or vapours were given.

The author wishes to express his cordial thanks to Prof. Dr. Y. Fukuda for his kind guidance and suggestions during the course of the investigation.

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The Functional Relationship between the Suction Intensity of Protoplasma and the Retention Capacity of Vacuolar Sap on the Plant Hydrature.*

By

Yasona Fukuda

(Recived Sept. 9, 1957)

Kramer (1933) found that killing root systems resulted in very large increases in the amount of water that moved through them. So it seems that in freely transpiring plants water is absorbed through the roots, rather than by the roots. Under the gradient of diffusion-pressure deficit extending from the atmosphere to the soil the loss of water from the leaf cells produces the development of a gradient of a diffusion-pressure deficit which is transmitted through a continuous, cohesive column of water and can there bring about the intake of water. The cohesive column, however, can not be formed in dead plant bodies, which begin to dry out at the ends fa thest from the bath water, for, unless plasma is living, plant bodies are not closed routes where water deficit must of necessity be compensated. Hoagland (1944) stressed the importance of healthy cells as essential to the maintenance of conditions required for continuous movement of water. It is thought nowadays that water moves in plant tissue along gradients of diffusion-pressure deficit. If transpiration removes water from the xylem of the leaf veins, a diffusion-pressure deficit is produced, which results in the movement of water f om the vacuoles, through the protoplasma membrane, and into the walls, although in the other way along the gradient developed across the cortex water moves from thes oil to the xylem. It is an evident fact that where there is a gradient of diffusion-pressure deficit between the two ends of a water system there is water movement, but it has not yet been proved that the osmotic pressure of the vacuolar sap and the diffusion-pressure of the soil are the two ends of a continuous water system. Forces of attraction between the osmotic pressure of vacuolar sap and the cohesion of water column in the xylem is not yet understood.

Kinoshita (1937) observed by cryoscopical method that the osmotic value of aerial phyphae of *Aspergillus itaconicus* Kinoshita was lower than that of culture medium. He explained that the swelling pressure of plasma caused absorption of water. A similar phenomenon was confirmed in my laboratory by Ikeshoji by plasmolytical method (1954) on lens moulds. We (Fukuda *et al.* 1953; Hayashi 1953) have found that with the swelling pressure of plasma the cells of xerophytic fungi can abso b water from air moisture. And Kubo found that the socalled artificially non-germinable pollen-grains

* This study is promoted in part by the fund from the Ministry of Education.
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of *Rhododendron* (1955) and *Compositae* (1955) and *Gramineae* (1957) were the air-inhabitants which germinated only on the stiff gelatin medium absorbing water from the air by the swelling pressure of plasma. I proposed at the 20th Meeting of Botanical Society of Japan in 1955 the general adoptation of the swelling pressure as the suction intensity factors of water in plants. It was a well known fact already at the time of Burgerstein that on the shoot pressed between news-papers to prevent the loss of water by stomatal transpiration the tissues at the growing point absorbed water from the grown-up tissues. I have affirmed that the former possessed lower osmotic value than the latter. So the suction intensity of plasma at the growing point might surpass that of the latter resulted from osmotic pressure. But those present at the symposium limited the application of my concept to the young cells, but, as to the matured cells they stuck to the old theo y. Before that, at the 19th Meeting, I had spoken that on a water cultured soybean plant with roots that were not well aerated the increase of osmotic value in the leaf cells compensated the decrease of swelling pressure of plasma which had been weakened by unsufficient respiration. At the 21st and 22nd Meeting I could state my opinion evolved on the interrelationship between the suction intensity of plasma and the retention capacity of vacuolar sap. I will give a full account of the series of our investigations in this paper.

Experimental Programme

Understanding that water moves in plant tissues along gradients of diffusion-pressure deficit, we have endevoured to find only the greatest deficit towards which water naturally flows. The osmotically active solution can absorb water from the adjacent solution or even from the atmosphere when the neighbouring media have smaller diffusion-pressure deficit. But even the vacuolar sap have no ability to absorb water even from the cohesive column of water if the deficit in it is not smaller than that of the vacuolar sap. Under the condition that the diffusion-pressure of the atmosphere is lower than that of the soil the diffusion-pressure of any upper ground portion of the plant is always lower than that of the soil, because the saturation of water is restricted by the gravity. It is not logical to suppose that the existence of the lower diffusion-pressure of water in the plant can be the proof of the ascent of water. The diffusion-pressure deficit theory consists of the unsolved problem that plant tissues have so high diffusion-pressure as still capable of saturation. As mentioned in the preface the negative pressure in the xylem can draw out water not only from the soil but also from the vacuolar sap. And the vacuolar sap, though it has infinite saturating capacity against solvent water, has no saturation deficit even against the cohesive column of water in the xylem if its diffusion-pressure is lower than that of the former. People must, therefore, find out the portion or the mechanism where or by which the

saturation capacity of diffusion-pressure becomes higher or more elevated than the water level physico-chemically determined against the gravity. To the solution of this problem it is desirable to adopt the direct representation of the water level, the concept of "hydrature" devised by Walter (1931) instead of the negative expression of water level, *i.e.* the saturation deficit of water or the concept of diffusion-pressure deficit. Walter's "Hydrature" is the intensity degree of water level. Water moves along gradients of decreasing hydrature. The intensity of moving should be proportionate to the hydrature difference between two points as denoted in Newton's law. My first study on hydrature was published in 1935. In the present paper I first fulfil the request of the late professor Dr. R. Kolkwitz by publishing the study of physical transpiration coefficient at different temperatures. But the greater portion of this paper may be dedicated to the late professor Dr. K. Shibata and also to the late professor Dr. W. Benecke whom I promised to complete my intended work. I regret that I can no longer express my gratitude to them, but to my great joy I have found that my old adviser Professor Dr. E. Schratz is active in his research work.

The drying curves of materials were drawn plotting the data got on several times experiments, so they formed smooth curves. From the analysis of these curves I reached the conclusion that the attraction forces between the osmotic pressure of vacuolar sap and the cohesion of water column in the xylem ought to be the swelling pressure of protoplasm in the plasma membrane of the cell. On living materials was measured the amount of respiration which is necessarily vigorous as long as plasma maintains swelling. In systematizing my idea on the water absorption of the plant, the members of my laboratory co-worked with me, taking part in diverse experiments. Some of their experimental results are denoted with their names in this paper.

Experimental results

Hydrature of free water

Exp. 1 Evaporation of the gravitational water and capillary water: As illustrated in the figure 1 the water over the filter papers in Petri dishes evaporated in a constant speed under the constant micro-atmospheric moisture deficit until the remained water was screened by the filter papers. The screened water evaporated also in a constant speed but more slowly than the former, until the remained water became hygroscopic water. The unscreened water over the filter paper is no other than gravitational water and the screened water in the filter paper capillary water. The decreasing curves of the total amount of these two states of water formed two straight lines, the former being steeper than the latter, intersecting each other at the transitional point (s_{30}, s_{15}, s_5) of two states. The hourly evaporation curves made step lines: u……s and s……h. The decrease of evaporation of the latter is not the result of the decrease of hydrature, but of

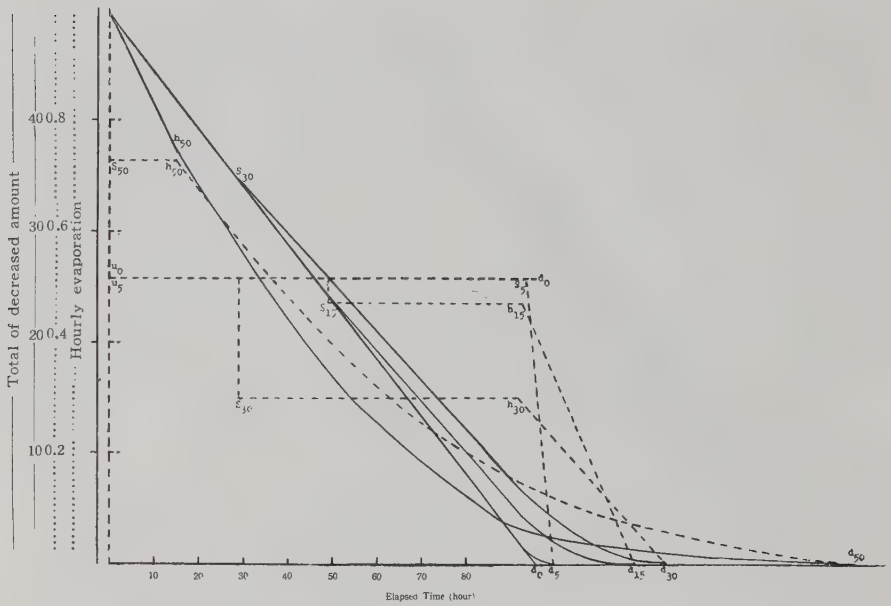

Fig. 1, Evaporation of imbibitional water at 30°C., R. H. 87 % in Petri dishes (2r=8.6 cm), screend by several sheets of filter paper (0, 5, 15, 30 and 50) written beside the marks:

u.....s: Unscreened water (water in bulk).
s.....h: Screened water (imbibitional).
h.....d: Hyproscopic in cellulose material to dryness.
Dotted lines: hourly evaporation.
Full lines: Totals of decreased amount.

the restriction of paper screen. But the drying curve of imbibitional water formed a logarithmic series (h·····d). From these results we may consider that when the water content approaches saturation capillary condensation, the hydrature of cell walls approaches 100%. In this state the water in the plant body is no other than free water. The amount of stomatal transpiration, therefore, may be assumed to correspond to the opening of stomata irrespective of the osmotic value of vacuolar sap. But at low contents the hydrature of cellulose cell wall may be similar to that of protoplasmic phase.

Hydrature of solutions

The figure 2 shows how, in the process of volume decrease, the hydrature of a solution changes whose initial concentration is 0.5 mol. As denoted in the table 1 the hydrature values were estimated after the Walter's table. The hydrature (Hy) of a solution after it has been con-

centrated may be calculated from the following formula:

$$Hy = 100 - q \text{ (Initial osmotic value)/Volume}$$

where 100 indicates the hydrature of saturated free water; volume the relative volume or the reciprocal of concentration; q the constant. The difference or real volume of solution does not concern the hydrature of the solution if its concentration does not change. Thus the hydrature of solution is a hyperbolic function of the osmotic value ascent caused by the volume reduction.

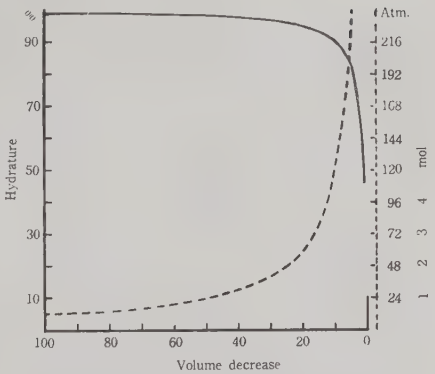

Fig. 2. Showing the relation of hydrature (full line) and molar concentration (broken line) or osmotic value (on the ordinate) of solution (initial 0.5 mol) in the process of volume decrease (on the absciss).

Table 1. Calculation of the hydrature of solutions in the process of volume variation

Mol of ideal solutions	Osmotic pressure in atm	Hydrature calculated by Walter	Decrease of volume	Hydrature calculated by the Formula (1)*	Decrease of volume	Hydrature calculated by the Formula (2)*
0	0	100		100		100
0.5	12	99.10	100	90.13		
1.0	24	98.19	50	96.26	100	98.26
2.0	48	96.48	25	96.52	50	96.52
4.0	96	93.05	12.5	93.04	25	93.04
8.0	192	86.5	6.25	86.08	12.5	86.08
16.0	384	75.0	3.125	72.16	6.25	72.16
32.0	968	48.0	1.563	44.32	3.125	44.32

* (1) .....$Hy = 100 - (12 \times 7.25)/V$ * (2) .....$Hy = 100 - (24 \times 7.25)/V$

General formula.....Hy = 100—q (Initial Osmotic value)/Volume.

Experiment 2 Evaporation of solutions: As illustrated in the figure 3 the amount of evaporation of the dilute solution is larger than that of the concentrated solution. The saturated salt solution separated solute crystals earlier than the sugar solution did. At any rate, as described in the table 2 the empirical formulae were got in the form of hyperbolic series. The hydrature change of the solution corresponds either to the volume decrease or to the concentration increase which was caused by the loss of solvent water through transpiration. The fact that the evaporation curve takes a similar form to that of the hydrature change suggests us that the

amount of evaporation corresponds to the degree of hydrature of the solution if the evaporation takes place under the constant external condition.

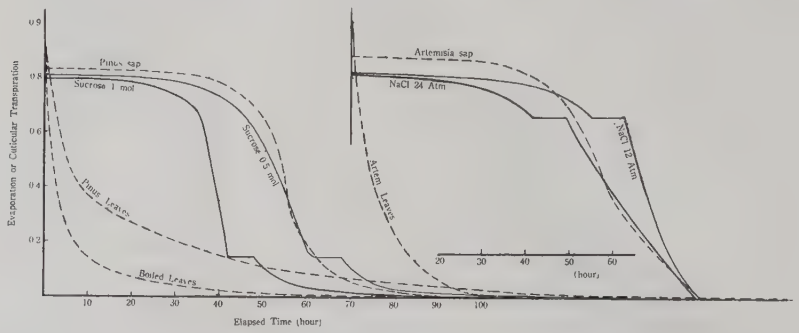

Fig. 3. The decreasing character of evaporation. Left: from solutions (initial 50 g) of sucrose (0.5 mol and 1.0 mol, on full lines) and expressed sap of *Pinus Thunbergii* Parl. (on a broken line); right: NaCl solution and sap of *Artemisia*. Do. Left: of cuticular transpiration of *Pinus* leaves (initial fresh weight 20 g and boiled 10 g); right: *Artemisia vulgaris* L. var. *indica* Maxim. leaves (initial 7.5 g) at 30°C., R. H. 87 %.

Table 2. Empirical formulae of hourly evaporation (in g.) curves of solutions

Pressed out sap of *Pinus Thunbergii* Parl.		(initial 50 g)	$Et=0.885-(20.2\times0.111)/(60-t)$
Sucrose Solution	0.5 mol	(,,)	$Et=0.847-(12.0\times0.195)/(60-t)$
Do.	1.0 mol	(,,)	$Et=0.847-(24.0\times0.098)/(50-t)$
Pressed out sap of *Artemisia Vulgaris* L. var. *indica* Maxim.		(,,)	$Et=0.930-(8.0\times0.313)/(60-t)$
NaCl solution	0.5 mol	(,,)	$Et=0.873-(12.0\times0.22)/(60-t)$
Do.	1.0 mol	(,,)	$Et=0.873-(24.0\times0.11)/(50-t)$

Generally $Et=A-\Delta q/(D-t)$

when $t=0$ ..... $E_0=$ Maximum $E=A-\Delta q/D$

$A=E_0+\Delta q\ D$

So the general formula is $Et=E_0+\Delta q/D-\Delta q/(D-t)$

where Δ: initial osmotic value in atm.
D: total duration
t: elapsed hours
q: specific constant

Cuticular transpiration of plant tissues

On this problem my precise study was published in the former paper (1935). I took a bulk whose volume was one cubic centimeter and the surface was ten square centimeter, and set it as the normal bulk on which the transpiration coefficient (k) might be converted to the normal coefficient (kn).

Experiment 3 Drying process of the potato tubers: As the comparative study I have selected potato tuber as control materials because any one can employ potato tubers in the normal state at any time of the year. Here for convenience sake I took 3 cm cubic sized pieces, and dried them at different temperatures. The ratio of surface development of this piece was 0.2 when it was compared to the normal. The results are illustrated in the figure 4. Until the water content became $1/27=3.7\,\%$ the curves at any temperature satisfied the exponential equation $W_t = W_0 \cdot e^{-kt}$; the magnitudes of the k values at various temperatures proportioned to the micro-atmospheric moisture deficit. Below the water content of 3.7 % the inclination of curves became slower, with almost the same value irrespective of the atmospheric temperature difference. It is expected (Gortner, 1937) that bound water shows a considerable resistance to the forces of evaporation. So people estimated the amount of bound water by the drying method. I found the drying speed of bound water is mostly self-determined owing to the resistance of the bound energy in absorbed films in plant gels, and the effect of the gradient of the atmospheric vapour-pressure deficit is scarce. There are two types of water in gels, loosely bound water which can be rather readily removed by pressure and closely bound water which is very resistant to removal. In this experiment (Fig. 4) the two types of water are recognizable. The former is the swelling water or the water which is held in a rather diffuse layer, and the latter, which is in another

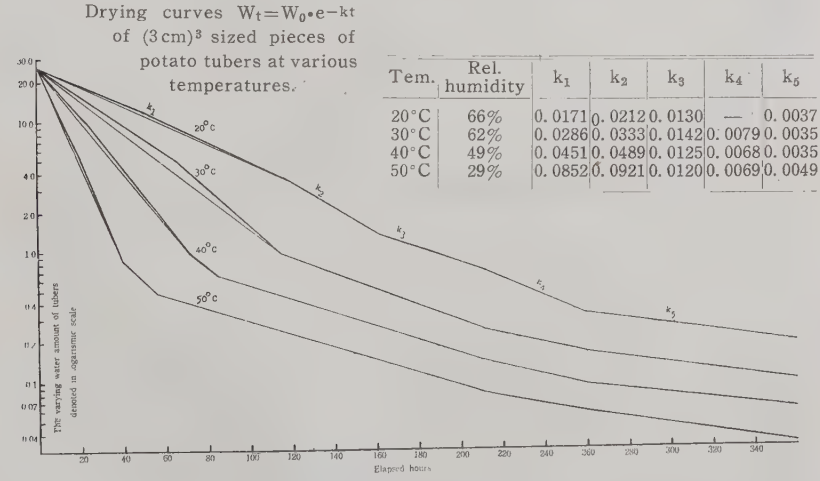

Fig. 4. Showing the drying speeds of swelling water of potato, caused by physical transpiration, k_1, k_2, which increase depending on the rise of temperature i. e. the increase of external moisture deficit; while the drying speeds of bound water, k_3, k_4, k_5, are almost constant without depending on the external conditions.

aspect the hygroscopic water is the closely bound water in an oriented shell of dipoles.

Expriment 4. The values of kn of different plants at different temperatures: Functional relations between transpiration coefficient (k) and the micro-atmospheric moisture dificit may be seen in the figure 4. But to demonstrate it clearly the k values got by many experiments are illustrated in the figure 5. The k values denoted in the figure 4 were five times multiplied to be converted as kn because the surface development of the material was 0.2. There is a straight-line relationship between kn values and micro-atmospheric moisture deficit in tuber-pieces of potato. On the leaves of pine-trees and camphor trees the relationships were not straight if the external condition extended to the moisture deficit of 50–60 mm. If the experiment is carried at below 40°C the linear relationship may be held on any plant part. This result coincides with the accepted belief that transpiration proportionates to atmospheric moisture deficit.

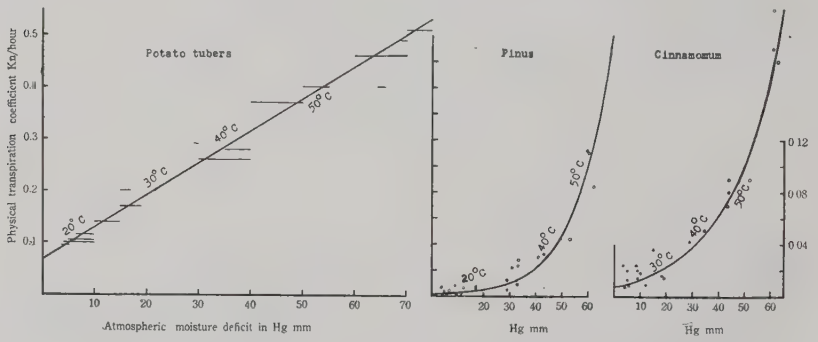

Fig. 5. Physical transpiration coefficient (k) of the normal sized plant body (n: 10 cm^2/1 cm^3): kn/hour in various atmospheric moisture deficit.

Left:	potato tubers	$kn = 0.07 + 0.006\,x$
Middle:	Leaves of *Pinuns Thunbergii*	$\log kn = -3 + (1/30)\,x$
Right:	Leaves of *Cinnamomum Camphora*	$\log kn = -2 + (1/50)\,x$

where x is Hg mm of moisture deficit

Heteroclitous-hydrature of swelling water and homeohydrature of solutions

In Fig. 4 of Exp. 4, the exponential curves of the drying process of plant tissues were denoted. According to mathematics the differential curves of exponential curves ought to be also exponential curves. Exponential curves were got for the decreasing process of the content of the plant by transpiration, so also for the variation of momental cuticular transpiration exponential curves may be got.

Expriment 5. Comparison of the drying curves of plant bodies and that of the expressed vacuolar sap: The hourly transpiration curves of the leaves of *Pinus Thunbergii* Parl. and *Artemisia vulgaris* L. var. *indica* Maxim. are

illustrated in the figure 3. They show typical exponential series. As we defined "hydrature" as the intensity moment of water level, the water movement, not only as liquid but also as atmospheric misture, ought to be proportioned to hydrature. Therefore, as it was affirmed that the cuticular transpiration of a plant body was proportioned to the water content of the body, it may be assumed that the water content means the relative water level of the body, i. e. the hydrature.

It was already mentioned in the former chapter that the decreasing process of hydrature of solutions caused by evaporation forms a hyperbolic curve. To prove that this relation equally holds good on the vacuolar sap of plants, the hourly evaporation curves of the sap of *Pinus* and *Artemisia* plants got by Walter's method were illustrated in the figure 3, besides those of sugar and salt solutions simultaneously experimented. From these data we can know that the decreasing process of hydrature of the vacuolar sap of the plants also follows hyperbolic series (Tab. 2).

The evaporation amount of the vacuolar sap decreases along the hyperbolic curve, so the hourly amount decreases in small quantity, while the momental cuticular transpiration of the plant body decreases in large quantity along the exponential curve. So in the figure 3 the data converted to the value on the 20 grams of fresh *Pinus* leaves and 7.5 g. of *Artemisia* leaves are illustrated. On the other hand 80 g. of *Pinus* leaves and 60 g. of *Artemisia* leaves were necessary to get 50 g. of expressed sap, respectively. In the case of boiled *Pinus* leaves, only 10 g. was enough to give the same amount of transpiration. Being boiled, the protoplasma of the leaf cells coagulated and the amount of free water in inter-cellular space increased, hence the transpiration did not decrease at the firist. But the curve was also an exponential form on the whole, though steeper than that of the fresh leaves. That the amount of cuticular transpiration is directly proportional to the water content is, therefore, caused by the common feature of protoplasma gel, dying or living. So long as the vacuolar sap and the plasma fluid remain unmixed in the dying cell, the water maintained in the vacuolar sap moves into plasma membrane, making up the swelling water deficit. So that the water is of the same value to the hydrature of plasma whether it is held in vacuole or in plasma itself, and the hydrature of the plant body is determined by the whole water content in it.

In accordance with the variable water content in plasma, the hydrature of plasma is very changeable. So the swelling water characterizes the plant as heteroclitous-hydrature, i. e. Walter's poikilohydral.

Contrary to the hetero-hydral feature of swelling water, the water state in solution is homeohydral, because the gradient of the curve (Fig.3) of the hyperbolic series (Table 2) decreases in very low-pitch more than half way; only near the end it suddenly falls. So that for quite a large part of the progress the vacuolar sap exudates water almost in constant pitch, endowing homeohydral feature to the plasma of the plants with developed vacuoles.

Suction intensity of plasma and retention capacity of vacuolar sap.

As discussed in the preceeding chapter the swelling property of a plant body is due to that of plasma gel either living or killing. But the dead plasma can not maintain swelling pressure long unless the body is immersed in the water. In the air dead plasma dries up sooner or later. So that for the maintenance of the swelling pressure, plasma of a plant body needs energy released by respiration.

Exp. 6 Osmotic value ascent caused by poor aeration: As illustrated in the figure 6, in both series, aerated and non-aerated, the osmotic value of the terrestrial portion of the plant ascended as the culture media concentrated. But the osmotic level is lower on the plants aerated than on the plants poorly aerated. Aeration was done for five minutes every day, and at that time the culture solution was saturated with oxygen. The media which were not aerated contained less oxygen than those aerated: 25–32% of oxygen was wasted in 2–6 atm. media, and only 10% in the highest 7 atm. medium in which growth was hindered. This osmotic ascent was not a temporary phenomenon caused by some metabolic disturbances. Rather the regular increase of osmotic level of the poorly aerated series suggests osmo-regulation against the decrease of the swelling pressure of plasma resulted from the poor aeration.

In the preceeding chapter we concluded that vacuolar sap sends water steadily and constantly into plasma whose hydrature easily decreases through transpiration. Then how can vacuolar sap recover the lost amount of water drawing it from plasma, when plasma itself has no independent force to suck water, for even though the hydrature of sap has become lower, that of plasma is still lower. To receive the lost amount of water in vacuolar sap from the plasma, the hydrature of plasma must become higher than that of the vacuolar sap.

Our present physiological knowledge allows us to speculate that plasma can evolve its swelling state to capture water in the lattice of plasma

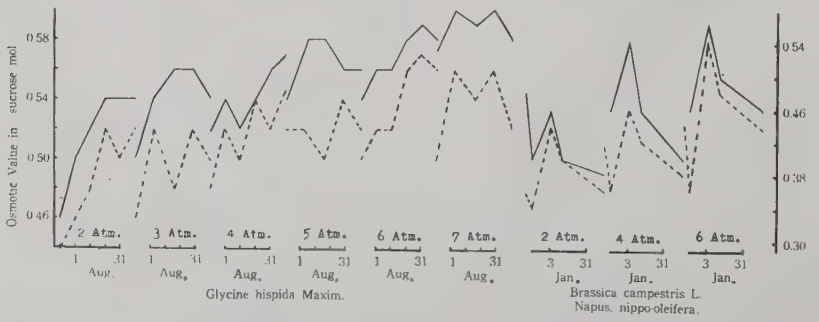

Fig. 6. Osmotic values of epidermis cells of plants in water culture, aerated (dotted lines) and non-aerated (full lines).

structure (Crafts et al. p. 63, 1949), and as this embraced water, by intermolecular forces, is loosely associated with the ordinary swelling water, it can be readily removed by osmotic pressure of the vacuolar sap. Thus the plasma, embracing only a small amount of water, can take high hydrature level, so we can estimate this function of plasma as the intensity momentum for absorbing water. Whereas the vacuolar sap, even when water is continuously supplied from the plasma, scarcely elevates its hydrature level, keeping for a long period lower hydrature level than plasma, and consequently accepts a large amount of water. So that we can estimate this function of vacuolar sap as the capacity momentum for absorbing water.

The present experiment proves that the high hydrature intensity is produced only by good aeration, *i. e.* by active force, so this feature may be termed as "suction intensity of protoplasma". And the feature of the vacuolar sap as "retention capacity of vacuolar sap". When the suction intensity of plasma is weakened by poor aeration, the water content of the vacuolar sap, lost through strong transpiration, can never be recovered. The ascent of osmotic value of the vacuolar sap thus occurs when the plant is poorly aerated, as observed in this experiment.

Effect of respiration on the water uptake in higher plants

The hydrature peak, from which the sucked water flows into vacuoles, takes place in plasma activated by respiration, which strengthens not only absorption of water in tissues but also transpiration.

Exp. 7 Transpiration of green plants under different gaseous conditions in media: Seedlings of green plants were aerated with oxygen or with

Table 3. Transpiration of green plant seedlings, formerly cultured three weeks in Knop's solution. Measured under the sunlight setting plants, roots in 300 cc Erlenmyer flasks, filled with water, kept at constant temperature in a water bath (This experiment was assisted by Shinji Takami).

Percentage of transpiration/h/one plant at 15.5°C.

A. *Glycine hispida* Maxim.

		hour of Nov. 14					hour of Nov. 15	
Sample	11~13	Water	14~15	15~16	16~17	Sample	Water	14~15	15~16
A	100	$+O_2$	71.4	80.7	45.6	A	$+O_2$	223.0	113.0
B	100	control	65.0	76.6	43.3	B	control	103.5	47.7
C	100	$-O_2$	33.1	60.3	41.2	C	$-O_2$	69.0	33.8
D	100	$+O_2$	101.5	85.2	48.1				
E	100	control	88.3	65.6	40.7				
F	100	$-O_2$	79.1	50.1	29.0				

B. *Zea Mays* L.

A	100	$+O_2$	131.5	103.3	43.9
B	100	$-O_2$	93.7	62.5	29.2

	May 13				May 14				May 15		
			14～16	16～19		9～10	11～15		9～11	12～14
C	100	$+O_2$	95.3	42.9	$+O_2$	82.0	128.0	$+O_2$	151.0	106.5
D	100	cont.	86.5	41.5	cont.	66.5	101.5	cont.	122.9	94.1

C. *Avena Sativa* L. (Victoria No. 1) at 20°C

	hour of Nov. 14					hour of Nov. 15					
	10～10		14～15	15～16		12～13		14～15	15～16	16～17	17～18
A	100	$+O_2$	69.5	46.7	D	100	$+O_2$	72.5	39.5	29.8	14.4
B	100	cont.	69.1	45.0	E	100	cont.	108.7	45.6	33.0	14.5
C	100	$-O_2$	86.8	47.5	F	100	$-O_2$	92.7	35.4	22.9	7.8

	Nov. 23			Nov. 24		
	13～15		15～17	8～10	10～12	12～13
G	100	$+O_2$	33.0	31.2	81.5	184.0
H	100	$-O_2$	42.7	23.3	60.5	163.0
I	100	$-O_2$	43.5	32.9	71.7	149.0

	Intermittent aeration May 1					Do. May 2						
	9～11		11～12	13～14	15～16			9～11	11～12	13～14	14～15	15～17
ABC	100	$+O_2$	125.0	55.6	99.6	EFH	$+O_2$	100	94.7	110.2	111.6	98.0
D	100	cont.	187.0	62.5	155.0	DG	cont.	100	68.9	103.1	101.5	94.25
E～H	100	$+N_2$	135.5	75.7	109.0	ABC	$+N_2$	100	102.6	104.6	96.5	99.53

$-O_2$ means aeration by nitrogen gas oxygen free.
$+ N_2$ means aeration by nitrogen gas not oxygen free.

nitrogen gas without oxygen, between the preparatory time and the test time. The data at the preparatory time were estimated as 100. The results are described in the table 3 and 4. The amount of transpiration increased a little more on the soy bean plants aerated ($+O_2$) than on the control C not aerated. On the plants treated with nitrogen gas, considerable decrease of transpiration was observed. The similar effect was also recognized on the corn plants.

But on the oat plants (Table 3, C) the effect of aeration on transpiration was not recognized, on the contrary the transpiration of the aerated

($+O_2$) plants decreased, probably owing to the disturbances of root system at the aeration.

It was precisely studied (Mitsui 1954) that rice roots secrete oxygen gas. Likewise on the oat plants it is not unreasonable to assume that the amount of oxygen necessary for the root respiration was supplied by photosynthetic assimilation in the aerial parts.

Exp. 8 Transpiration of green plants whose root respiration was restricted by chemical inhibitors: This experiment was conducted by Takeshi Takaoki. As the respiratory inhibitors he used potassium cyanide, 2.4-dinitrophenol, sodium arsenite and phenyl urethane. When the cotton plants and buckwheat plants were treated with the effectual amount of inhibitors, within the limit of unharmful concentrations, there was a remarkable parallelism among the restriction of root respiration, the decrease of transpiration amount of the air portion and the increase of the osmotic value of cells. Only the daily progress of cotton plants treated with three kinds of inhibitors is illustrated in the figure 7. All of the data are converted into the percentages against the uninhibited value 100.

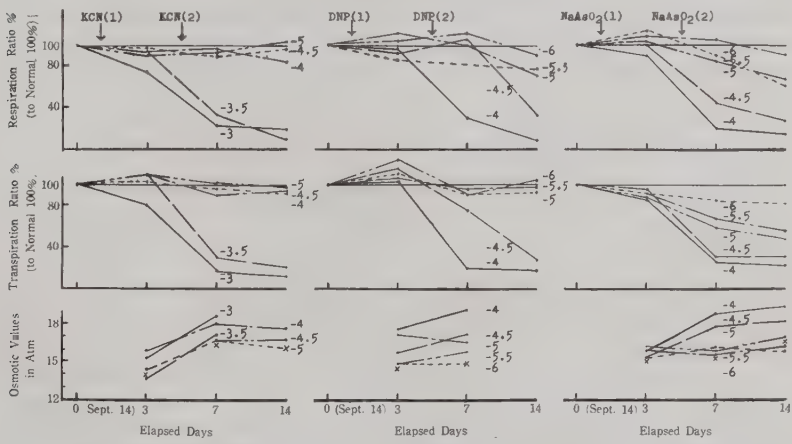

Fig. 7. The corresponding decrease of transpiration (on the middle line) and elevation of osmotic value (lower) contacted with respiratory inhibitors, potassium cyanide, 2, 4-dinitrophenol and sodium arsenite, on *Gossypium indicum* Lam. seedlings cultured in Knop's solution.

Osmotic values were measured at the incipient plamolysis of epidermis cells. All other values are the percentages of measured values against the assumed normal values of the same plant, which also estimated as percentages against 100, the observed values of the controls.

Arrow mark (1) denotes the one-tenth medication and (2) the full medication. Minus numerals on curves denote the log of the inhibitor mol. concentrations.

Equilibrium between the hydrature peak of plasma and the hydrature level of vacuolar sap

There was a parallelism between the ascent of osmotic value of cell sap resulted from the maturation of vacuolar differentiation and the aging of plasma (Fukuda 1952; Fukuda and Kaku 1952, 1953) accompanied by the retardation of respiration. This increase of osmotic value is not only the adaptation to such aging of plasma, but also to the reserve of nutrient solutes. We have observed that this continuous increase of the osmotic value was interrupted at the flowering time, perhaps because of the removal of solutes from the vacuoles.

Exp. 9 The abrupt drop of osmotic value at the flowering time on the normal and the early matured soy bean plants: Shosuke Kaku treated the soy bean plants photo-periodically. When the plant was treated in early period they entered the reproductive differentiation very early while the osmotic ascent in the vegetative growth was not yet so large. The early matured treated plants had, on the same day, slightly higher osmotic value than the others. But the plants which were in bloom possessed lower osmotic value than the others as denoted in the table 4. The decrease of osmotic value means the elevation of hydrature level, to which plasma keeps equilibrium, resulting in the accerelation of respiration.

Table 4. Osmotic value of soy bean plants in KNO_3 mol, sown on June 17, grown in the soil, kept 80 % moisture of the water-holding capacity.

Treatment of short day (12 h)	Osmotic value at the time of observation					
	Flowering*			Podding*		
	July 1	July 22	Aug. 7	July 7	July 28	Aug. 28
Treated in early stage	0.24*	0.30	0.32	0.28*	0.31	0.37
Do. in late stage	0.26	0.27*	0.34	0.26	0.33*	0.36
Control (natural)	0.25	0.28	0.31*	0.25	0.30	0.40*

At the flowering period of rice plants and of barley plants (Mitsui 1954) the oxidation power of roots changes to the reduction power. This phenomenon suggests us that at the flowering period the oxygen produced from photo-synthesis is utilized for respiration there. So we assume that the decrease of the osmotic value in vacuolar sap made the plasma keep its hydrature level higher by enforcing the respiration to keep the equilibrium with the sap.

Exp. 10 The measurement of the respiration and the osmotic value on glucophytic and halophytic plants: We have already affirmed the occurrence of osmotic value ascension corresponding to the depress of respiration: on soy bean plants and rape plants in Exp. 6, on cotton plants and buck wheat plants in Exp. 8. In 1937 I found on glucophytic plants osmotic

value ascended against the medium concentration along a convex curve, while on halophytic plants along a concave curve. Using the same species as I used, Takaoki reaffirmed those results and measured the amount of respiration on the plants cultured in media of various concentrations. The results are summarised in the table 5. On the glucophytic *Plantago major* the osmotic value rose steeply in the range of comparatively dilute media, while on the halophytic *Plantago colonopus* slowly in that media. And in the 15 atm. medium the two ascending curves intersected. Qo_2 of root respiration was low at the high osmotic values of *Plantago major* and high at the low osmotic values of *P. colonopus*. Salt tolerable *P. colonopus* absorbed water easily by the swelling pressure of plasma strengthened by strong respiration, and possessed low osmotic value, which resulted in maintaining young state of plasma. Salt intolerable *P. major* hardly absorbed water because respiration was restricted in concentrated media, and possessed high osmotic value, which made the plant unadaptable to concentrated media.

Table 5. Respiration of root, measured by Warburg manometer, denoted as Qo_2/200 mg fresh weight/h in 4 cc medium, hypertoned with NaCl added on nutrient solution, in which plant grew normally. The osmotic value of the pressed sap of leaves of the plants was cryoscopically determined (June 13~14).

		Concentration of medium (in atm.)					
		2.6	5.2	7.8	10.4	13.0	15.0
Plantago major (Glucophyte)	Osmotic value of leaves (atm.)	5.2	22.0	30.0	33.0	35.0	39.0
	Qo_2 of roots	2.25	3.0	2.8	2.7	2.6	2.2
Plantago colonopus (Halophyte)	Osmotic value of leaves (atm.)	17.0	20.0	24.0	29.0	31.5	39.0
	Qo_2 of roots	4.0	4.25	5.1	5.3	5.3	5.3

The extent of the active swelling space of plasma for hydration

Reinders (1938), Commoner (1943), and van Overbeek (1944) found that auxin increased the water intake of potato tissue. Reinders found that water was absorbed only by well-aerated tissue. It was believed that auxin (Reinders 1938; van Overbeek 1944; Kramer 1949; Crafts *et al.* 1949), malate (Commoner 1941) and very dilute chloroform (Wilson and Kramer 1949) increased respiration which released energy utilized in uptake of water. The fact got by Commoner, (1943) that a decrease in wet weight by tissue immersed in aerated hypertonic sucrose solution could be prevented by the addition of auxin, rather the wet weight increased where KCl or fumarate were also present suggested me that respiration, accelerated by auxin, actively enlarged the swelling space of plasma overcoming the super osmotic force of hypertonic medium; while Kobayashi, Hatakeyama and Ashida (1956) found that in *Avena* coleoptile the water uptake did not

increase in auxin solution where osmositics are contained sufficiently enough to cause incipient plasmolysis in the plant, but the water uptake of the plant increased in a hypotonic solution with auxin.

Reexamining these worker's results I conclude that by utilizing the respiratory energy plasma can develop the active swelling space in the range between the hydrature of pure water and that of solution isotonic to the cell sap. The result of the preparatory experiment to testify my conclusion will be shown below. We cultured rape plants, *Brassica campestris* L. *Napus* var. *Taina*, in soils in different percentages of water holding capacity, and estimated the amount of respiration of the leaf-stalk with Warburg manometric apparatus in which 0.1 cc of various concentrated bath solution hypertoned with the unmetabolizable lactose was put, which touched the lower end of each test piece. And we knew that the drought tolerable plants respirated more intensely than the intolerable ones, and the respiration of plant tissues rose proportionately to the increase of the concentration of bath solutions. But the trend of respiration increase turned to the decrease before the concentration of the bath solution became isotonic to the cell sap. The amount of respiration ought to correspond to the plasma activity to extend active swelling space for hydration. Following is one of the data obtained in this experiment.

Exp. 11 Respiration of *Brassica* leaf-stalk in media with various omsotic values, conducted by Takeshi Takaoki: The test pieces were taken from the plants cultured in the soil with different moistures, as described in the table 6. though tap water was used for the bath solution, the amount was only enough to prevent desiccation of materials in the Warburg vessels. So the measured respiration may show how active the plant is to keep the active swelling space. The data showed that the plant tissue in dry soil respired more.

Table 6. Respiration (30°C) of leaves of *Brassica campestris* L. var. *Napus* (var. *Taina*) estimated on 5~8 pieces (surface area 2×2, 18 cm^2) in Warburg manometer with 0.1 cc water added.

Soil moisture, % of W. holding cap.	60	38	24	15
Qo_2/dry weight/mg/h	2.7	2.9	3.4	4.5
Qo_2/fresh weight 10 mg/h	2.6	3.3	4.4	7.0

The active water absorption displayed by leaf temperature: Newton (1925) found that a plant in hypertonic solution respired more than that in hypotonic solution. The similar result was obtained by Takaoki as mentioned in the preceding chapter. The comparison of the respiration of two naturally different plants, rooted in the soil, is very troublesome, so we measured leaf temperature difference as the manifestation of respiration.

Exp. 12 Leaf temperature difference (Leaf—Air) **of cotton plants cultured in different moisture contents:** This experiment was conducted by Minoru Honda. The result was denoted in the table 7. At noon expected results were got. In the sun leaf temperature became higher than the atmospheric temperature. The cooling effect of stomatal transpiration was efficacious when the hourly amount surpassed 12.5 mg per 1 cm² leaf area. And more transpiration was seen only on the plant grown in 80~100% moisted soil. On the plant in the dry soil, leaf temperature was higher than the micro-atmospheric temperature. But at mid-night no appreciable transpiration took place. But the leaf of the moist soil plant was cooler while that of the dry soil plant was warmer than the micro-atmospheric temperature. The lower the soil moisture was, the higher the leaf temperature was, though there was no difference in the water loss. Subsequently the drier the soil was the more energy the plant needed for the water uptake.

Table 7. Showing the elevation of the leaf temperature (on Sep. 27~28) of 85 days old xeromorphic *Gossypium* plant, cultured in different moisture content kept every day by irrigation to sandy loam in pot.

Moisture percentages of max. water-holding capacity	At noon			At midnight		
	Transp. mg/cm²/h	Stomata opening	Temperature difference	Transp. mg/cm²/h	Stomata opening	Temperature difference
100%	13.5	72	−0.3°C	1.0	15	−0.1°C
80	12.5	82	0	1.0	14	−0.1
50	9.5	80	+0.2	0.5	12	+0.2
20	9.5	34	+0.4	0.5	8	+0.4
13	5.0	16	+0.6	0.5	8	+0.9

The temperature was measured electrically.
The degree of a stomatal opening was measured by Darwin porometer.

Exp. 13 Leaf temperature of derooted shoots: I expected that the leaf temperature of the derooted shoots might become higher than when it was

Table 8. Cooling effect of transpiration on the upper shoot of *Helianthus tuberosus* L. its lower part was cut off.

		Transpiration (mg/dry weight 1 g/10 Min.) and leaf temperature difference from the air (Leaf—Air)							
Shoot out of the water A	Transpiration	204		198		194		184	150
	Temp. (Leaf-Air)	−0.6°C		−0.8		−0.6		−0.4	−0.2
	Transpiration	53	52	44	38	30	22		
	Temp. (L.-A.)	+0.4°C	+0.2	+0.4	+0.3	+0.3	+0.1		
Shoot in the water B	Transpiration	77	64	61	60	57	56	55	54	50
	Temp. (L.-A.)	−0.3°C	−0.3	−0.2	−0.3	−0.3	−0.1	−0.1	−0.3	−0.3

rooted, because they had no new water supply. The result of the experiment of Minoru Honda is denoted in the table 8. The leaf of the plant B whose cut end was kept in the water was cooler than the micro-atmospheric temperature, while the leaf of the plant A which also was cooler even after it was picked out of the water, transpiration becoming more vigorous than when it was inserted in the water, suddenly became warmer than the micro-temperature in accordance with the abrupt drop of transpiration: the 53 mg, transpiration could not cool A, while the less amount was effective for B without exception. To give the same effect on A more than 150 mg. transpiration was needed.

The fact that more transpiration was needed to cool A than B shows that the temperature of A was higher than B, consequently A respired more than B. So that it is affirmed that plants need suction energy to absorb water. In other words, plants need energy to maintain the state of diffusion-pressure deficit.

Osmophilous features and respiration of lower plants immersed in medium

On the lower plants immersed in medium passive absorption by transpiration does not take place. Their water absorbing process is thought to be a relatively simple one of osmosis, the living cells simply providing the differentially permeable membranes necessary for its functioning. But we have found, in this case also, the necessity of more respiration and respiratory materials to maintain growth in hypertonic solutions.

Exp. 14 Growth of acid fast bacilli in the hydrature gradient

This study was performed by Takamasa Nishio, and the abstract of the results are illustrated in the figure 8. The names labelled on the curves indicate the chemicals added for hypertoning. The ends of the curves coincide with the possible limit of viability resisting low hydrature, which are determined cryoscopically. The viability limit of the non-pathogenic acid fast bacillus strain S 50 B (found in down water) in different media was found in the following downward order, glycerin> con. nutrient medium> $NaCl > KCl > NaNO_3$>sucrose > glucose > KNO_3. Growth quantity was measured by using electrophotometer. The generation time in the media of

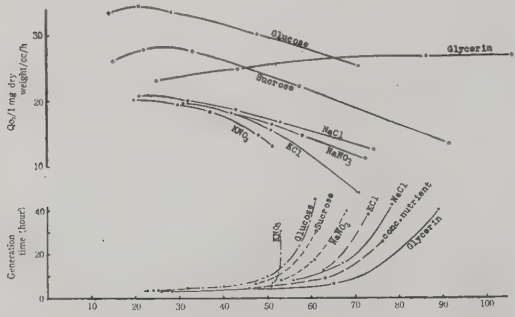

Fig. 8. The relation between respiration (upper) and growth (lower) of a osmophilous sewage strain of acid fast bacillus (S 50 B) in hydrature gradients of various solutions. The names on curves denote osmositics in solutions.

50～60 atm. was in the same order, from shortest to longest, as viability limit.

Judging from the order of viability limit and the generation time it can not be said that harmful ion actions existed in salt medium, because growth was rather better in salt medium except KNO_3 than in sugar medium except glycerin. With Warburg manometer respiration amount was measured: to 0.2 cc of condensed bacillus suspension, whose dry weight was 0.00124 g., 0.6 cc of 1/5 M phosphate buffer solution and 0.2 cc of 20% glycerin were added. The total volume of bacillus suspension was 1 cc. To this 1 cc of hypertoning chemical solutions were added. Respiration was strong in the medium where growth was speedy. In the media of the same concentration the respiration amount was larger in glycerin and sugar than in salt. As this strain is more salt torrerable than Timothy strain or human-type pathogenic bacilli, osmotic equilibrium may be maintained as in halophytes by allowing the penetration of salt ions into cells in the hypertonic salt medium, though that does not occur in the hypertonic organic medium, in which the additional active work ought to be necessary to maintain hydrature equlibrium.

According to Sakai (1956) sugars were not metabolized by the acid fast bacilli till glycerin was totally consumed. So that glucose and sucrose were neither absorbed to elevate osmotic value nor utilized for respiration, and though the bacillus consumed much of the initial glycerin in the hypotonic solution, in a little hypertonic solution the viability suddenly lessened rather more greatly than in salt medium. But as glycerin was the most favorable carbon source for the growth and the maintenance of viability, the amount of respiration, which enabled this bacillus to grow under the 89 atm. condition, enabled the same bacillus to maintain its viability under the 160 atm. hypertonic condition.

Exp. 15 Increase of the respiration of blue-green algae in hypertonic medium: This experiment was done by Shigeru Nakatani. The result is denoted in the table 9. *Oscillatoria irrigua* grown on the coastal rock was isolated by the enrichment method and cultured in the standard Benecke's solution. *Microcystis robusta* grown on the cliff of estuary was also isolated

Table 9. O_2 consumption of 5 mg fresh colony of *Oscillatoria irrigua* in medium 2 cc hypertoned with NaCl added.

O_2 consumption: mg/2 cc/h

Elapsed hour	NaCl volume mol.	0	0.1	0.3	0.5	0.8
0 ～ 1 h		27	18	29	38	49
1 ～ 2		28	16	20.5	25.5	23.5
2 ～ 3		23	11	15.5	20	19
3 ～ 4		21	11	15	18	17
av. 2 ～ 4 h		22	11	15	18	19

Do. of *Mycrocystis robusta*

	0	0.15	0.4	0.75
0 ~ 1 h	0.75	2.75	0.6	4.0
1 ~ 2	−0.3	−0.4	−0.2	−0.2
2 ~ 3	0.05	0.075	0.25	0.25
3 ~ 4	0.25	0.225	0.3	0.45
4 ~ 5	−0.1	0.15	−0.125	0

by the enrichment method but using salty solution. The viable limit in salty solution was 0.1 mol to the former and 0.4 to the latter, respectively. Respiration was measured at 37°C with Warburg manometer: a piece of colony, fresh weight 5 mg., was put in 2 cc of phosphate buffer solution added with NaCl.

This glucophytic *Oscillatoria irrigua* was injured after one hour in over 0.1 mol NaCl. But as long as the alga was viable, respiration increased as the concentration of NaCl increased (as the vapour pressure in the vase was low it did not influence the measurement of the O_2-pressure). On *Microcystis robusta* during the first one hour and 2–4 hours respiration was markedly large in hypertonic solutions. But sometimes respiration dropped; perhaps this halophytic alga regulated osmotic value at that period by absorbing salt ions in the body.

Discussion

The results on the kinds of water in plants will be discussed.

1) **Water still retained at 365°C in the form of absorbed films in gels:** Firmly bound water in solid with more than 1.0 density (Nelson and Hulete 1920).

2) **Water intimately associated with the hydrophilic sols and gels:** Consequently immobilized.

3) **Water intimately associated with the lyophilic colloids:** No longer available to act as a solvent. Fukuda (1933) noticed the physiological meaning of hexahydrate of sucrose.

4) **Water embraced by intermolecular forces of colloids:** Water molecules bound in an oriented shell of dipoles and very resistant to removal.

5) **Bound Water or hygroscopical:** This water is very resistant to be vaporized. In the present experiment (Fig. 4) the drying speed was determined by its affinity to colloid, almost independent of air moisture deficit.

6) **Loosely bound water rather easily removable:**

7) **Swelling water of plasma:** Molecules coordinating to a lattice structure of plasma.

8) **Imbibitional water:** Shull (1913) noted that the imbibition pressures (*e. g.* of the seed colloids) are much greater than the maximum osmotic pressure. But when the tissue absorbs enough water to cause osmotic

action of the vacuolar sap, imbibitional force does not surpass osmotic force. Anderson and Kerr (1943) found that slightly vacuolated young cells of cotton bolls could absorb water even from wilted plants. He supposed this was because the high imbibitional forces of cells resulted in development of much higher diffusion pressure deficits than would be expected from the osmotic pressure of their sap. But Stocking (1945) reported young apical leaves of squash remained unwilted, although their diffusion pressure deficit appeared to be lower than that of the wilted older leaves.

I also noticed every summer that young apical parts of a morning glory remained unwilted and flowered, absorbing water from wilted bodies derooted. I explain that these portions full of plasma, having stronger active suction intensity, absorbed water from the leaves with well developed vacuoles.

9) **Solvent water of solutions in vacuoles:** Water can move along gradients of increasing osmotic pressure, *i. e.* from the higher hydrature to the lower. Though cohesive column of water consists of solute-loss water, even high osmotic pressure can not draw water from it if its diffusion-pressure is lower than that of the solutions.

10) **Cohesive column of water:** The diffusion-pressure deficit originated in this column by transpiration causes passive absorption of water from the soil. But if we neglect the existence of active forces necessary to maintain the right to demand the loss of water to be saturated, our explanation on the water relation is not logical. Without this force the water column can not be in a connecting system of vacuolar sap and soil moisture. In the artificial pattern, a pipe filled with water can serve as a part of this column. A siphon can conduct water, if the upper end is lower than the upper water level, though, at any other point of the tube, it can be higher than the upper level or lower than the lower level. In this water system water flows along gravitational orientation, but in the plant system water is sucked up against the gravitational orientation. In the former case the upper end of the connecting route, siphon, must not be higher than the upper water level; in the latter case the lower hydrature end of the connecting route, cohesive column, must not be lower than the lower hydrature level of the vacuolar sap.

11) **Water attracted in the lattice of plasma:** According to the old theory, only the semipermeability of protoplasma membrane, but never the attractive force, was taken in the explanation of the vacuolar sap attracting cohesive column. I propose, the water attracted in plasma lattice by active energy liberated by respiration does the function of the head flow of a suction pump. Water in the xylem coheres into column by cohesive force, but the vacuolar sap and cohesive column must be connected in one water system by this attraction force.

12) **Capillary water:** If a well-watered plant is surrounded by a moisted atmosphere, its cells become nearly turgid and its hydrature nearly 100. Then the water in the intercellular spaces may become like water held by

capillary attraction. The capillary takes the part of a screen, and though the screen depresses the speed of transpiration, that speed continues constantly for a long while, as in water in bulk.

13) **Hydrature of the plant**: These different water forms do not consist of different water molecules but of the same molecules. And the hydrature of the plant is determined by the total sum of water content, which gives different values according to the phase in which water movement takes place. When the plant is saturated with water, though there is a resistance to gravitation to deduct the hydrature, the hydrature approximates that of capillary water. But as a result of water loss by transpiration, the water undergoes the changes in kind or form continually. The water in the transpiring plant takes the form of swelling water in plasma. When the water amount available for the swelling of plasma is removed, the remained amount takes the form of bound water and finally the embraced water by intermolecular forces.

In the increasing process of water in plant, (*e. g.* in the germination proccess) conversely, imbibed water adheres to the absorbed film in plasma, associating in dipole shell or with the hydrogen bond. When the plasma colloid is saturated with the bound water, the plasma is physico-chemically activated. Then the layer of water molecules may form bridges between the backbones of adjacent polypeptide chains to produce a vein of strongly bonded water separating the two chains by the width of a water molecule (Crafts *et. al.* p. 66). The separated width corresponds to the width of water layers, which increases as the bonded water amount increases, resulting in an increase of swelling water if the water supply is sufficient.

The necessity of respiration for the physiological water absorption

The physico-chemical swelling functions mechanically not physiologically in the following relation:

Swelling value − Plastic force = Hydral value

where hydral value = Swelling pressure employed for water uptake
= work to absorb water

In solution, the hydral value can not rise above the diffusion-pressure of medium, so the decrease of plastic force does not result in the increase of hydral value, only it enables the swelling value to decrease. Therefore the increase of the employed swelling pressure occurs only when the plasma is laid in hypotonic solution. Corresponding to the increase of the employable swelling pressure in hypertonic solution the swelling value increases physico-chemically. But to surpass this swelling value respiration was necessary to liberate energy for the work, as we have shown in our experiment, in higher and lower plants. It proved that the higher the concentration of culture medium was the more the respiration increased.

The mechanism of the space making for tne elevation of swelling value:

As described in the article before last, swelling pressure enlargement was done by the separation of polypeptide chains spreading the interspace

between chains, resulting in the increase of swelling space. This physico-chemical enlargement of swelling space is stopped when the swelling value becomes equal to the external medium. The physiological space making may also be done in the same mechanism, but with energy, overcoming the static hydration limitation. The energy liberated by respiration may widen or separate still undivided polypeptide chains, spreading also the side chains and releasing the cross-linking of the chains. Utilizing energy, no matter how wide and how numerous the "reaction chambers" of Sponsler (1940) and Warburg may evolve, the reaction space will not react, and remain unsaturated against hypertonic medium, only keeping hydrature equilibrium between them. I will speculate that, as the active work of energy, micro-Brownian motion of side chains and hydrophile radicals arise which shifts hydrogen bonds and make the chamber the field of water attraction or the dynamic field of water bondage. According to Stewart (1939) the coordination of water increases with the shift of structure. Because of the increasing energetic agitation the possibilities for contacts between different molecules increase immensely and the tendency toward bonding becomes greater.

The water held by micro-Brownian motion in micro-capillaries should be fairly free. I do not claim that the cytoplasm actually secretes water into vacuoles. Romell (1918) and Köhnlein (1930) suggested that the tension resulting from transpiration stimulates the root cells, causing increased active absorption. In our experiment it seems probable that the osmotic value of vacuolar sap is controlled by the active absorption of plasma, which results in the maintenance of hydrature equilibrium, the latter playing the role of bridges between them.

The categories of heterohydrature and homeohydrature are given by Walter (1931), the former for the feature of the lower plant whose hydrature varies according to the external moisture, and the latter for the higher plant which is viable with osmo-regulation against the external moisture change. I found that the heterohydral feature is orignated from the swelling property of plasma, while the homeohydral from the osmotic property of vacuolar sap. And the swelling ability of plasma takes the part of suction intensity and the osmotic ability of the vacuolar sap that of retention capacity for the water. These two momenta should not be taken for the two components of the hydration magnitude. The product of the intensity value of the plasma and the retention value of the vacuolar sap is meaningless. Plasma and vacuolar sap are two different phases. Plasma has its own hydration magnitude which is the product of the diffusion-pressure deficit and the water amount of it. And the vacuolar sap has also its own magnitude obtained in the same way. Only they have different momenta on function. Statically the intensities of the two phases keep equilibrium, but dynamically the developed vacuole has greater water capacity than the plasma, while the plasma can surpass the vacuolar sap in its suction intensity.

The vitalistic view point before Pfeffer (1877), the spongiole theory of de Candolle (1832) and the turgor-pressure theory of Hofmeister (1863) are

out of question. Opposed by the mechanistic theory of osmotic pressure, the vitalistic conceptions got by the studies of sap exudation and root pressure have not yet been generally recognized. These phenomena should be reconsidered from my view point. Bose (1923, '7, '8) and Molish (1929) observed in plant shoots rhythmic pulsation corresponding to the variation of water uptake. But their vitalistic view points also were not generally recognized. Of course physiologists are forced to explain the behavior of cells, how some cells operate like suction pumps and others like reduction valves of force pumps. But Pfeffer (1897), Wieler (1893), Lepeschkin (1906), Ursprung (1929) and Frey-Wyssling (1929) reduced these functions of cells to the differenciated plasma-permeability. I recognize their theory of differenciation of cell organs, but not on permeability but on swelling.

The parallelism of respiration and water uptake were observed by Newton (1925), Henderson (1934) and others. But plasma may expend energy to maintain living properties. Heyl (1933) presumed electro-osmotic potential is maintained by respiration. This phenomenon, together with the active solute uptake, interests recent workers, such as Commoner and Thimann (1941), Krogh (1946) and Mitsui (1954). If the adequate supply of solutes is provided in cells by respiration, then water ought to be taken up by the increased osmotic pressure of the cells. Before them Atkins (1916), Priestley (1920, '22) and Crafts and Broyer (1938) had already established the idea though without knowledge of respiration. Although a renewed "secretion theory" (Kramer) was claimed by Maximov and Lominage (1916), Bennet-Clack *et al.* (1936), Mason and Phillis (1939), Lyon (1942), Went (1944) and others, their opinion was based on the plasmolytic-cryoscopic discrepancy (Crafts). Cells rich in greater non-solvent space (plasma) produces less condensed pressed sap than the cells with developed vacuoles (Atkins 1916, Walter 1931, Fukuda 1952).

Though Styles (Crafts p. 113) and others thought of the swelling, the adopted this concept only to the colloids in vacuolar sap. Walter (1931) noticed the importance of plasma swelling for they water uptake, but he, studying *Fucus* sp., concluded that swelling pressure of plasma obeys the osmotic pressure of vacuolar sap. The recent writers pay attention to how much water a plant takes by passive transpiration and how much by active osmotic mechanism. Even if they notice the phenomena observed by Kinoshita (1937) and us, they will only add to the two forms of water uptake, passive and active, another form of absorption by swelling pressure which occurs only in young tissues.

As noticed by Levitt (1947), the energy released by respiration in a plant body may be insufficient to maintain all the water in the plant. I dare not say all water is directly absorbed by the swelling pressure of plasma. In my opinion vacuolar sap in cell takes water into itself from a little higher head flow of the plasma membrane which absorbs very little water steadily from the adjacent cells in which abundant easily removable water is retained by osmotic force. Though vacuoles are numerous in a plant, they are solitary organs. But by continuous system of plasma

water is relayed from vacuole to vacuole.

The swelling pressure of plasma should be equal to that of sap at turgid state of cells. And in proportion to the increase of hydrature deficit the swelling pressure becomes higher than the osmotic pressure, the increase of difference stops before the plasmolytic threshold. At the incipient plasmolysis the swelling pressure becomes lower than the osmotic pressure. So the shrink of plasma begins at the threshold (Fukuda 1933).

Conclusion

This series of study will give a new explanation to the water movement in plants. The evaporation of water screened by filter papers was similar to that from capillary water but not from living cell walls. The living cell walls consist of colloids and swell with plasma membrane in a similar way. Plasma then can take up by its active suction intensity the water in the cellulose membrane. Tomojiro Kaibara, in our circle, studied the air life of yeasts. Mucous substances, the degenerated product of cell membrane, swells and condenses air moisture around the membrane. According to his personal information he has observed, through an electron microscope, the mucous substance covering the aerial hyphae of lens-moulds. Thus aerial inhabitants which absorb moisture through their whole surfaces have moisture condensers outside the cell walls.

In higher plants, vitalists believe, endodermis actually absorbs water from the soil. Outside the endodermis the vacuolar sap of cortex cells catches and stores water which come along the hydrature gradient from the soil through the cellulose walls of the tissue. Endodermis, agitated by respiration, and its active suction intensity being hightened, absorbs this water and sends it into the xylem. So the diffusion-pressure inside the endodermis, though lower than in the soil, is higher than not only in the xylem but also in the cortex, as affirmed by Ursprung (1932). Though the diffusion-pressure of the water in the xylem becomes low by transpiration, the water not only coheres into a cohesive column but also attracts the swelling water of plasma of the whole body. Being plulled down, the diffusion-pressure of the swelling water, physico-chemically should become equal to the water in the xylem, but physiologically it holds its normal height, which obeying the equillibrium law elevates the hydrature in the xylem, causing deficit, which wants saturation. The amount to be saturated is called "suction force" by Ursprung and Blum or "diffusion-pressure deficit" by Meyer (1938, '45) or "hydrature deficit" by me. The hydrature in plasma is very variable, *i.e.* heterohydral. By strong transpiration it descends more easily than that of vacuolar sap, and draws water out of the vacuolar sap and from the soil. But if the plant is well watered the hydrature height of the plasma is recovered and the plasma sends water into the vacuolar sap, until the equilibrium between them is reached. This work of plasma which consumes energy liberated by respiration, is the

motive power to sustain the hydrature deficit in the plant.

Summary of the results

I have studied the variability of the kind and form of the water, originated in the differentiation of cell organs, and the unity of states among their functions, which are affected not only by the molecules in the phase but also by all the water molecules in the plant system.

1. The property of water in different forms was described in discussion.

2. The route of water movement in the plant is described in conclusion.

3. It was proposed that the motive force of water uptake is neither "passive" transpiration nor "active" osmotic pressure but the swelling pressure of protoplasma, whose hydrature is physiologically elevated higher than the other two.

4. The function of plasma is elevating suction intensity in plants, and that intensity controls osmotic value of the vacuolar sap.

5. Vacuoles enlarge the capacity for water retention. And owing to the homeohydral feature of solutions the vacuolar sap can supply water almost at a constant hydrature level.

6. Plasma can not retain so much water as vacuolar sap, but receiving water from the vacuolar sap the plasma can escape the severe decrease of its hydrature, and thus in spite of the hyterohydral feature of plasma, the higher plant becomes homeohydral.

7. Plants can not utilize solar energy expended for transpiration; plants must work to take up as much water as was lost by transpiration.

8. The physiological enlargement of plasma swelling is made possible by energy expenditure liberated by respiration. Many experimental data are illustrated: on higher plants:—variously aerated on roots, respiration partially restricted by inhibitors, poorly watered by restricting irrigation, derooted; on lower plants:—blue-green algae in hypertonic medium, acid fast bacilii in hypertonic medium.

9. It it assumed that the swelling pressure of plasma is of equal to the osmotic value of the vacuolar sap in the turgid state. With the increase of diffusion-pressure dificit the former becomes larger than the latter, but begins descending before the incipient plasmolysis, and at the threshold it is lower than the latter.

10. Owing to the strong respiration in plasma, the osmotic value of vacuolar sap is low in active young cells, and the ascension of osmotic value is accompanied with the aging of plasma; conversely, the osmotic value descent occurs with rejuvenation.

11. Xeromorphous and halomorphous plants, which respire more strongly, are more viable in lower hydrature than mesomorphous plants.

12. The exponential formulae, empirically studied by me in 1935, are the theoretical expression of hydrature variation accompanying the decrease of water content in plants. The variation of cuticular transpiration obeys this law. The exponent k is a constant which not only proportionates to the surface development but also to the external diffusion-pressure deficit.

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The Influence of Density on the Dry Matter Production of *Fagopyrum esculentum*.

By

Hideo Iwaki

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Introduction

It is quite obvious that one of the most interesting and fundamental problems in plant ecology is to study how the density of a plant community can affect the growth of each constituent plant, and, consequently the dry matter production of the plant community. This problem has also been tackled for a long time by many agriculturists as well as silviculturists, because of its importance for obtaining the highest yields of food, timber, etc. There are, therefore, already a number of studies concerning this problem; among them the classical and comprehensive study of Clements et al. (1929) on a plantation of sunflower and wheat is particularly well known. Recently, Kira et al. (1953) discussed clearly the relationship between the yield and the density of plant communities, using a simple empirical equation which was deduced from the data of their experiments with some field crops.

These studies have produced a lot of descriptive results in each experimental case, or some empirical law on the relation of plant growth as a whole, or of the yield of some special crop, to the density, but so far they have provided but little information on the actual process and mechanism which cause the differences in the growth of plant communities with varying density. In order to obtain such fundamental ecological knowledge, we should at first analyse the phenomena on the one hand into the dry matter production of the constituent plants, and on the other hand into the environmental factors which have been changed by the growing plants and will affect the plant growth in the next step, throughout the growing period (see Monsi & Saeki 1953).

In the present paper, the author intends to make clear the relationship between the density and the growth of plant communities with various densities, by means of the analytic-synthetic method which was initiated by Boysen-Jensen in his classical work, "Die Stoffproduktion der Pflanzen, 1932," and has been followed by such investigators as Romose (1940), Larsen (1941), Monsi & Saeki (1953), etc. The factors which are acting on the growth of the plant in the community, i.e. photosynthesis, respiration, ratio of non-photosynthetic systems to photosynthetic systems, and light

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conditions in the community, etc., will be discussed in detail, on the basis of the experimental data. Thereafter, the growth curves of communities with various densities will be synthetically composed with the factors thus analyzed.

It may also be expected that the information derived from the present investigation, which has mainly been carried out on a simple artificial plant community of buckwheat, will prove effective for the logical explanation of a complicated ecological phenomena occurring in natural fields. On the other hand, it should also provide basic knowlege which will be useful in determining the most effective density of crop plants and trees for obtaining the highest yield.

Experimental Results

In order to study the effects of varying density on the dry matter production of a buckwheat community, a series of experiments was performed in 1954 and 1955, in the experimental field of the Toride Upper Secondary School, in South Ibaraki.

In both years carefully selected summer buckwheat seeds (mean seed weight 30mg) were sown in regular square disposition early in June. Prior to the sowing, the field soil was well fertilized. Three different grades of spacing, i.e., a spacing of 5cm., 10cm. and 20cm. in both directions, were employed so that the density was 400, 100 and 25 plants per square meter, repectively. On the sampling days ten or twenty plants within each plot were measured for the dry weight of each component organ (leaves, stems, roots and reproductive organs). The sampling was started on 6 June for the 1954 experiment, on 2 June for the 1955, respectively, and further sampling made at constant intervals of 7 days for about two months. So far as the present experiments were concerned, it could be supposed that the original numbers of plants in each plot remained unchanged even in the later stages of development, so it was possible to calculate the standing crop per 1 sq. m. by multiplying the mean values for plant weight by 400 for the 5cm. plot, 100 for the 10cm. and 25 for the 20cm. one, respectively.

Growth in plant weight: The effects of varying density on the growth in plant weight are set out in Tables 1–a and –b. It is evident that among three plots (5cm., 10cm. and 20cm.) there are significant differences in mean plant weight (oven dry weight) and that these differences become more marked as the plants develop. On 26 July 1954, a mean dry weight of 1.59 g. was measured at the highest density plot as compared to 4.72 g. for the medium and 13.96 g. for the lowest (a ratio of 1 : 3.0 : 8.8). For the 1955 experiment, the corresponding figures, determined on 23 July 1955, were 1.56 g., 5.44 g. and 19.60 g., respectively, and the ratio was 1 : 3.5 : 12.6. These results, then, indicate that increasing density causes a marked suppression of growth in individual plant weight.

Table 1-a. Effect of varying density on the mean dry weight of buckwheat plant. (1954)

Date	Days after sowing	5 cm.	10 cm.	20 cm
June 21	14	0.041	0.041	0.040
28	21	0.090	0.139	0.172
July 5	28	0.206	0.452	0.696
12	35	0.492	1.33	2.21
19	42	1.03	3.13	6.45
26	49	1.59	4.72	13.96
Aug. 1	56	1.67	4.67	15.31

Table 1-b. Effect of varying density on the mean dry weight of buckwheat plant. (1955)

Date	Days after sowing	5 cm.	10 cm.	20 cm.
June 11	9	0.050	0.047	0.041
18	16	0.177	0.177	0.224
25	23	0.577	1.02	1.82
July 2	30	0.599	2.94	5.99
9	37	1.05	3.80	9.87
16	44	1.37	4.57	11.80
23	51	1.56	5.44	19.60

The variations of standing crop with time, calculated from the data of Tables 1-a and 1-b, are shown in Fig. 1. The time trend is the same at all densities, showing exponential growth in earlier developmental stages, but nevertheless between densities there are marked differences in growth rate during the same period; the highest growth rate of standing crop is observed at the lowest density. The differences in standing crop which result from altering the density become progressively smaller with the development of the plants, although they are still significant. For the experiment in 1955, the maximum standing corp of 622 g./sq.m. was measured on 23 July 1955 at the highest density, while the corresponding figures for the medium and the lowest densities were 544 g./s q.m. and 490 g./sq.m. respectively.

Growth in leaf area: The depressive effects of higher density on the dry matter production of plants can be largely attributed to the higher degree of self-shading of leaves in the population. It was therefore decided, as a next step, to follow the variations in leaf amount in the course of plant development at each density. The variations with time in leaf area index (Monsi & Saeki's Blattarealschicht; total leaf area of population per unit land area) and the relative light intensities at ground level are brought out in Fig. 2. From the results of the 1955 experiment (Fig.

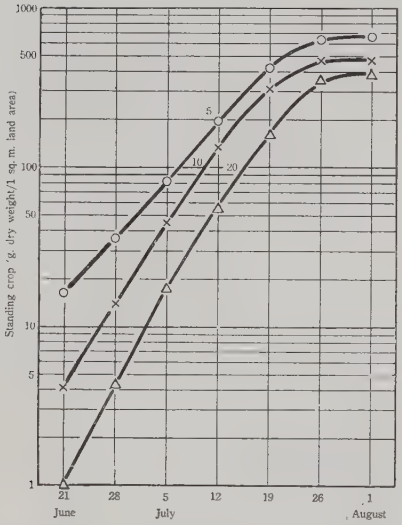

Fig. 1-a. Variation of standing crop with time (1954); g. dry weight per 1sq.m. land area. Spacings between plants: 5cm., 10cm. and 20cm.

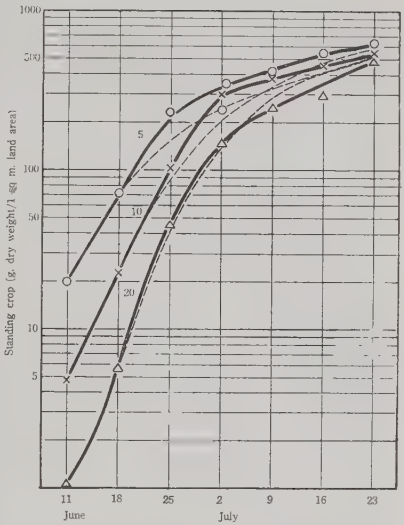

Fig. 1-b. Variation of standing crop with time (1955); g. dry weight per 1sq.m. land area. Broken lines show calculated values of standing crop.

2-b) it can be seen that at the highest density, the magnitude of leaf area index has reached 4.0 by late June, while in the most widely spaced plants, the figure is still low (1.2). The difference in leaf area index is also reflected in the variation in relative light intensity penetrating to ground level; on 25 June 1955 at the lowest plant density about 32 per cent of full daylight still reached the ground level compared with the relative light intensity of only 5 per cent for the densest planting. Under these conditions it may be expected that the depression of CO_2-assimilation by leaves has become important by this time in the lower leaves of the high density population. Further analysis of this aspect of the problem will be presented in the following section.

At all densities leaf area index increases rapidly at first, then becomes more or less constant. Maximum leaf area index was attained in all plantings by late July (42 days after sowing) in the 1954 experiment, and early in July (30 days after sowing) in 1955. Among plots with different densities there are no marked differences in the final value of leaf area index, but nevertheless the highest values of leaf area index are in general observed at the highest densities; in the 1954 experiment the final leaf area index observed for the 5 cm., 10 cm. and 20 cm. plots being 3.7, 3.1 and 2.8,

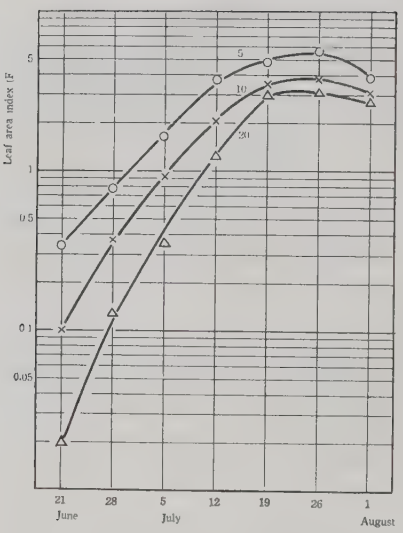

Fig. 2-a. Variation of leaf area index with time in 5 cm., 10 cm. and 20 cm. buckwheat stands (1954).

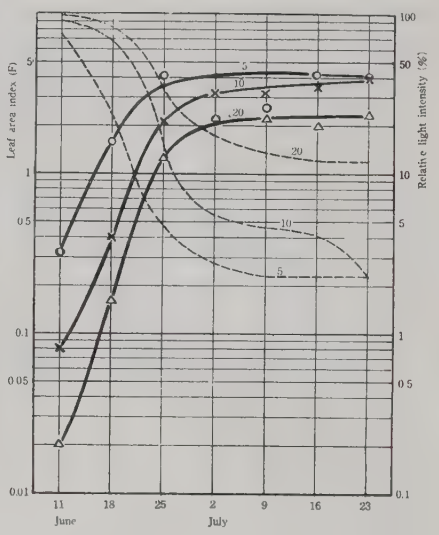

Fig. 2-b. Variation of leaf area index with time in 5 cm., 10 cm. and 20 cm. buckwheat stands (1955). Broken lines show relative light intensity at ground level (%).

and the corresponding figures in 1955 being 4.0, 4.0 and 2.3, respectively.

Although it is not yet clear why the final leaf area index of the 20 cm. plot was considerably smaller in the 1955 experiment compared with those of the 5 cm. and 10 cm. plots, it might be presumed that water economy is concerned with this result.

Net assimilation rate (NAR): Net assimilation rate, which defines the efficiency of the leaf as a producer of new materials, is represented by the following equation (Gregory, 1917):

$$\mathrm{NAR} = \frac{1}{F}\frac{dW}{dt} \qquad (1)$$

where F is the total leaf amount of the plant and dW the increase in dry weight in a period of dt. NAR has been used in growth analysis of field crops by Blackman and Wilson (1951), Watson (1952) and others.

Fig. 3 shows the time trends of NAR (leaf weight basis, g./g. dry weight/week) for three plots of buckwheat plants, calculated from the data of 1955. There is a significant reduction in NAR with increasing densities at the earlier developmental stages; during the period from 18 June to 25

June 1955, when the NAR of each plot attained its maximum, values of 3.86, 7.28 and 10.60 were determined for the 5 cm, 10 cm. and 20 cm. plots, respectively.

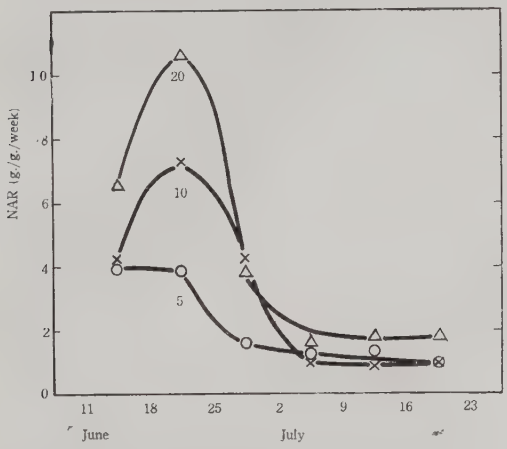

Fig. 3. Effects of varying density on NAR (g. per 1 g. leaf weight per week), calculated from the experimental results in 1955.

Although NAR provides a useful means of assessing the productive efficiency of plants under a given condition, it does not provide a means of elucidating, on a quantitative basis, the way in which each of the environmental factors might operate in determining dry matter production in a plant community. For this reason, it is necessary to make further analysis of NAR with respect to the factors which are acting onplant growth, i.e. photosynthesis, respiration, ratio of non-photosynthetic systems to photosynthetic systems, etc.

In Equation (1), the term dW, the increase in dry matter, can also be expressed as follows:

$$dW = F(a-r) - C \cdot r_c \qquad (2)$$

where F and C represent the total amount of photosynthetic (leaves) and of non-photosynthetic organs (stems, roots etc.), respectively, a and r the rate of assimilation and respiration per unit weight of leaves, and r_c the intensity of respiration of non-photosynthetic organs in unit weight. So, assuming that the value of dt is unity, Equation (1) can be transformed in the following way:

$$\mathrm{NAR} = \frac{F(a-r) - C \cdot r_c}{F} = (a-r) - \frac{C}{F} \cdot r_c \qquad (3)$$

This formula shows that the greater the value of $(a-r)$ the higher is the value of NAR, and on the other hand, the greater the value r_c or C/F, the lower is the value of NAR. Therefore, it becomes necessary to discuss the relationship between the degree of density and the magnitude of each of the three quantities, $a-r$, r_c and C/F.

Net assimilation: The term $(a-r)$ in Equation (3) respresents the mean daily net assimilation per unit weight of leaves in the stand. As the magnitude of $(a-r)$ is not directly measurable, an indirect method of

estimating it was employed.

As the first step in this calculation, a daily light assimilation curve of a leaf was constructed, similar to that of Monsi & Saeki, by the combination of the light assimilation curve of the leaf (Fig. 4), determined by Boysen-Jensen's method, and the daily course of illumination. Fig. 5 shows the daily assimilation curve on fine summer day in our latitude constructed from the hourly light assimilation curve of a leaf in the 5 cm. plot (Fig. 4, Curve I).

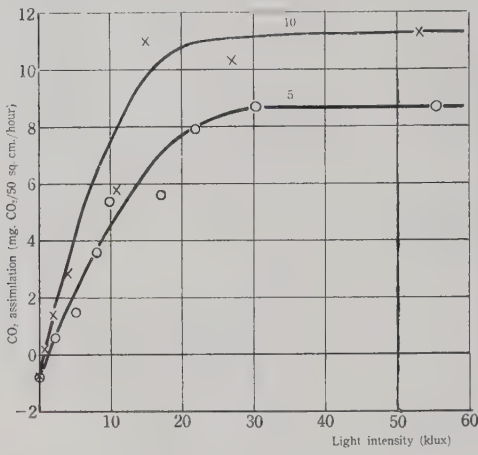

Fig. 4. Hourly light assimilation curve of a buckwheat leaf in 5 cm plot (I) and in 10 cm plot (II); mg. CO_2 per 50 sq. cm. leaf area and hour.

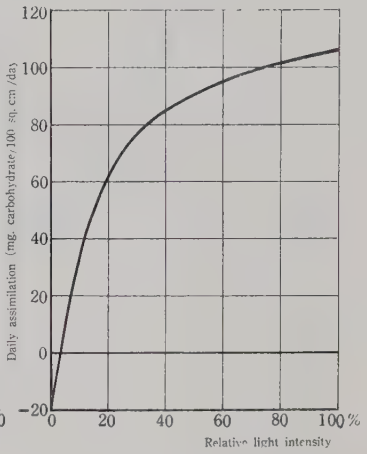

Fig. 5. Daily light assimilation curve of buckwheat leaf in 5 cm. plot, on fine July day; mg. carbohydrate per 100 sq. cm. leaf area and day.

For the indirect estimation of the total assimilation in a buckwheat stand, we must determine the relative light intensity which the leaves in the plant community receive, as well as the above mentioned daily light assimilation curve.

Concerning the light condition in a plant community, it has been demonstrated by Monsi & Saeki that the logarithm of light intensity under the leaf canopy decreases linearly with the increase in leaf amount, and that the relationship between these factors can be expressed by Beer-Lambert's law, as follows:

$$I = I_0 \, e^{-KF} \qquad (4)$$

where I represents the light intensity under the leaf canopy, I_0, the incident light intensity, F, the leaf area index and K, the extinction coefficient. It becomes clear, also, that K is almost constant for the same plant species

and the value of K is determined by the arrangement, inclination and transmissibility of the leaves. In the case of buckwheat plants, K was estimated to be 0.9, because the leaves are distributed horizontally and their light transmissibility is about 8% (see again Monsi & Saeki, p. 39). The estimated value of K was also found to coincide with the result obtained by direct determination. From Equation (4), therefore, the relative light intensity I (%) under the leaf canopy becomes 40.5%, 16.4%, 6.7%, 2.7%, 1.1%..., when $F=1, 2, 3, 4, 5,...$, respectively.

In order to make the calculation easier, it was assumed that the buckwheat plant community is composed of several leaf planes, all of them without blank portions and that the uppermost leaf plane receives full daylight, and the relative proportion of full daylight received by the 2nd, 3rd,... leaf plane is 40.5%, 16.4% and so on.

By the combination of the relative light intensity and the daily light assimilation curve (Fig. 5), we can calculate the daily assimilation of buckwheat stands with different values of leaf area index. As seen from Fig. 6, the daily assimilation of the stand increases with increasing leaf area index, but the rate of increase falls off with the increase of leaf amount, as the result of self-shading of the leaves.

From the results of Fig. 6, we can easily calculate the magnitude of $(a-r)$ related to leaf area index, assuming that 1 g. dry weight of leaves corresponds to 400 sq. cm. leaf area. The results obtained are illustrated in Fig. 7. It was found in general that the magnitude of $(a-r)$ falls considerably as the leaf area index increases.

As mentioned above, the leaf area index in the denser plots was much larger than that of the sparser plots in the earlier phases of development. So it may be said that the magnitude of $(a-r)$ in the same period is significantly larger in sparser than in denser plots. For instance, on 11 July 1955, when the leaf area index in the 5 cm., 10 cm. and 20 cm. plots was shown to be 4.1, 2.1, and 1.2, the value of $(a-r)$ was 0.25, 0.37 and 0.42 g./g./day, respectively. But these marked differences in the value of $(a-r)$ tend to decrease with the development of plant communities, because the differences in total leaf area among them become smaller in the later phases of development. On 16 July 1955, for example, the value for leaf area index was 4.2 for the 5 cm., 3.5 for the 10 cm. and 2.4 for the 20 cm. plots, and, therefore, the value of $(a-r)$ became 0.25, 0.30 and 0.36 g./g./day, respectively. These results correspond fairly well with the results of Fig. 3, in which the NAR of three plots is compared.

In the above discussion, all the buckwhat leaves are assumed to have the same rate of photosynthesis, regardless of the density of the stand. However, the maximum rate of photosynthesis of the buckwheat leaf was found to be affected by planting density; i.e. the maximum rate of hourly assimilation of 8.6 mg. CO_2/50 sq. cm. was measured for the 5 cm. plot; while for the 10 cm. plot, the rate of 11.3 mg. CO_2/50 sq. cm./h. was observed (Fig. 4). The real magnitudes of $(a-r)$ in sparser plots must, therefore, be

greater than the above values which were calculated from the lower value of the hourly assimilation of the denser plot (5 cm.).

Respiration of non-photosynthetic organ: Equation (3) shows clearly that the greater the intensity of respiration of non-photosynthetic organs, r_c, the lower the magnitude of NAR. To determine the relationship between the intensity of respiration and the planting density, the output of CO_2 by respiration was measured for stems, roots and reproductive organs

Table 2. Loss of dry matter by respiration of nonphotosynthetic organ (mg. $C_6H_{10}0_5$/g./day). (1955)

Plot		June 16	June 25	July 4	July 11	July 18
5 cm.	stems	99	109	27	20	15
	roots	75	51	13	14	14
10 cm.	stems	—	116	52	43	—
	roots	—	43	16	27	—
20 cm.	stems	—	137	73	27	—
	roots	—	61	11	19	—

of the buckwheat plant with Boysen-Jensen's apparatus at 25°C. The results obtained are summarized in Table 2. The rates of respiration are given in mg. carbohydrate, $(C_6H_{10}O_5)_n$, per 1 g. dry weight of each plant organ.

As seen in the Table, the rates of respiration of non-photosynthetic organs were generally high in the earlier stages and low in the later stages of growth.

The differences in the intensity of respiration at different densities were not so marked, except in the case of stem.

C/F ratio: As shown in Equation (3), the C/F ratio, the ratio of the non-photosynthetic system to the photosynthetic system, also operates in determining the magnitude of NAR through its influences on the extent of

Table 3. Variation with time of C/F ratio in 5 cm., 10 cm. and 20 cm. buckwheat plantation (1955).

Plot	June 11	June 18	June 25	July 2	July 9	July 16	July 23
5 cm.	0.51	0.71	1.64	3.20	3.90	5.17	5.99
10 cm.	0.53	0.53	1.11	2.30	3.12	3.76	4.02
20 cm.	0.52	0.49	0.67	1.32	3.03	4.00	5.45

dry matter loss by respiration. Table 3 shows the time trends of the C/F ratio, calculated from the results of the 1955 experiment. The ratio of C/F becomes greater with the mature plants at all densities as a result of

the increasing dominance of the non-photosynthetic system over the photosynthetic system. In the earlier stages (18 June–2 July, 1955), the C/F ratio tends to be higher with increasing density, but in the later stages no apparent correlation between them is observed. Therefore, it may be said that the relative proportion of dry matter loss by respiration to the net assimilation is higher in closely spaced stands than those in widely spaced.

Thus, it may be concluded that the higher NAR values of widely spaced stands in the earlier stages of growth are due to 1) the higher relative light intensity in the plant community, 2) the higher rate of photosynthesis of the leaves themselves and 3) the lower value of the C/F ratio in comparison with the closely spaced stands.

Comparison between directly measured and calculated standing crop of *Fagopyrum esculentum*

In the previous section, the author has discussed in an analytical way the influences of density on the production of matter in a plant community. Such analysis indicates that the relations of density to 1) the light conditions in a plant community, 2) the photosynthetic ability of the leaves and 3) the C/F ratio are chiefly responsible for the differences in NAR found among the three differently spaced buckwheat stands. In order to give further support to this conclusion, the author calculated the dry matter production of each buckwheat stand, using these relations as a basis, and tried to construct theoretically the growth curve of the standing crop of each stand and then to compare the calculated values with the observed.

Concerning the calculation of dry matter production in plant communities, some work has already been done by Romose (1940) for a moss (*Homalothecium sericeum*), and Larsen (1942) for an annual plant (*Solanum nodiflorum*). Larsen calculated the dry matter production of stock of *Solanum nodiflorum* from both the dry matter gain through assimilation by the leaves and the dry matter loss by respiration of leaves, stems, roots and reproductive organs, and found that the estimated values agreed rather well with the direct measurements. In this caculation, however, the *dry matter reproduction* of the photosynthetic and non-photosynthetic systems was not considered. For the sake of resynthesis of the process of plant growth, extending over a fairly long time, as the logical image, one must bring the reproduction as well as the production of dry matter into calculation, because the growing plants are always increasing their photosynthetic and non-photosynthetic systems and carrying out an expansive reproduction of dry matter. In the present work therefore, the dry matter production of a buckwheat stand was estimated on the basis of its photosynthesis and respiration, and, then, the dry matter reproduction was computed on the basis of experimental data.

In order to calculate the dry matter production in a plant community,

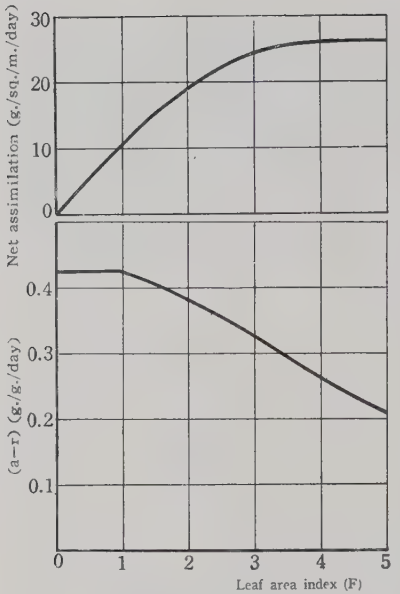

Fig. 6. (Upper). Daily net assimilation of buckwheat stand (5 cm), related to leaf area index, on July fine day; net asimilation is expressed as g. carbohydrate per 1 sq.m. and day.

Fig. 7. (Lower). Mean daily net assimilation per unit leaf weight, related to leaf area index; g. carbohydrate per 1 g. leaf weight and day.

it is necessary first to know the daily assimilation. The daily assimilation is decided not only by the magnitude of leaf area index but also by the daily weather (mainly light condition and temperature). The daily course of illumination at Toride was measured with an electric photometer (Toshiba No. 5) and the curves obtained were divided into three weather types (fine, light-cloudy and rainy-cloudy). Three typical types of the daily sequence of illumination are illustrated in Fig. 8. Daily assimilation-leaf area index curves can be constructed, in the same way as in Fig. 6, by the combination of these three types of daily illumination and hourly light assimilation curves of buckwheat leaves. The hourly assimilation curves shown in Fig. 4 were used as a basis for this calculation, curve I being used for the assessment of the daily assimilation for the 5 cm. plot and curve II for both 10 cm. and 20 cm. plots. From such curves as Fig. 9, the daily assimilation per unit area of buckwheat stand can be readily assessed, if the value of the leaf area index is given and the type of weather is indicated.

As the starting point for the calculation of dry matter production, the amounts of leaves, stems and roots observed on 18 June 1955, in the buckwheat stands were used. For example, in the 5 cm. plot, the dry weight of leaves, stems and roots was found to be 41.5 g./sq. m. (leaf area index = 1.59), 24.2 g./sq. m. and 5.1 g./sq. m., respectively. It was rainy on this day. So it can be determined by Curve III of Fig. 8 that the net assimilation in the 5 cm. plot on this day was 14.0 g. carbohydrate/sq. m.

As shown in Equation (2), daily net production (dW) can be represented as the difference between daily net assimilation and daily respiration by non-photosynthetic organs. Daily respiration is given as the function of the activity of respiration and the amount of each non-photosynthetic organ. The rates of respiration of stems and roots in each stages of development are given in Table 2. For example, the rate of respiration on 16 June 1955, per 1 g. dry weight of stems and roots was deter- mined

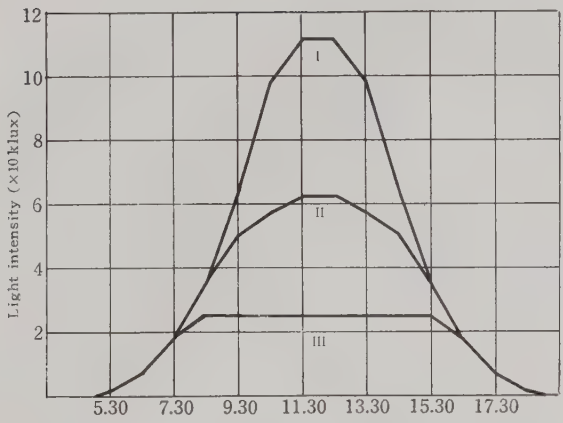

Fig. 8. Daily marches of illumination in July at Toride. I, II and III show the typical curves of illumination on fine, light clouldy and rainy cloudy days, respectively.

1924). The corrected value of stems and roots carbohydrate g./day, respectively. As shown before, the dry weight of stems and roots per 1 sq. m. land area of the 5 cm. plot was 24.2 g. and 5.1 g. on 18 June 1955, the daily respiration loss of dry matter was therefore assessed to be ca. 2.2 g. and ca. 0.4 g. carbohydrate/sq. m. respectively. The daily gain in dry matter per unit area of the 5 cm. plot, therefore, can be calculated as follows:

$$14.0-(2.2+0.4)=11.4 \text{ g./sq. m.}$$

The increments of dry matter produced in this way are distributed according to a certain ratio to individual organs and are used for the development of new leaves or for increase of stems, roots or reproductive organs. The author refers to this ratio as the "*distribution ratio of dry matter*." Although the distribution ratio of dry matter is one of the most important factors acting on the reproduction in a plant community, related to be 0.099 and 0.075 g. carbohydrate / day at 25°C in the 5 cm. plot. Before application of these data determined at 25°C to the assessment of the dry matter loss by respira- tion of 18 June 1955, they must be corrected to the mean tempera ture (24°C) of that day. As the basis for the temperature correction of the respiration rate, the author employed the tempera ture-respiration curve of leaves of *Solanum tuberosum*(Lundegårdh, was 0,092 and 0,070 g.

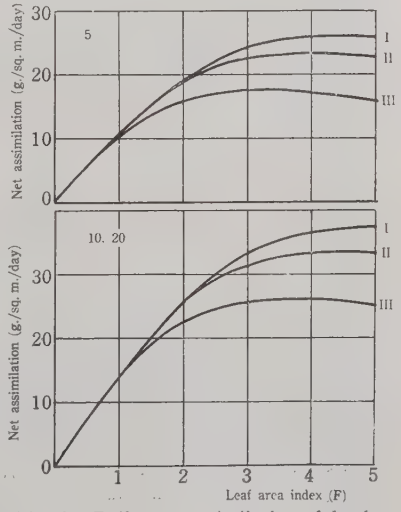

Fig. 9. Daily net assimilation of buckwheat stands, related to leaf area index (F), on a fine day (I), light-cloudy day (II) and rainy-cloudy day (III) in July at Toride.

tively little is known with regard to the relationship between the distribution ratio and the environmental factors.

In the 1955 experiment, the distribution ratio of the dry matter increment was calculated for individual organs, on the basis of the experimental data. Table 4 shows the variation of the distribution ratio

Table 4. Distribution ratio of increased dry matter to the individual organs (%) (1955).

5 cm. plot	June 11–June 18	June 18–June 25	June 25–July 2	July 2–July 9	July 9–July 16	July 16–July 23
leaves	56%	19%	11%	2%	0%	0%
stems	38	67	73	54	28	30
roots	6	9	10	9	4	3
reproductive organs	—	5	6	35	68	67
total	100	100	100	100	100	100
10 cm. plot						
leaves	65	43	22	6	7	9
stems	28	46	63	59	18	6
roots	7	8	9	12	12	7
reproductive organs	—	3	6	23	63	78
total	100	100	100	100	100	100
20 cm. Plot						
leaves	68	59	35	0	0	0
stems	25	32	49	61	50	42
roots	7	7	11	23	25	27
reproductive organs	—	2	5	16	25	31
total	100	100	100	100	100	100

in each of the three stands. The proportion of dry matter distributed to the leaves is noticeably high at first, and more than half of the whole gain of dry matter is utilized for increase of leaves at all densities, but such utilization decreases rapidly as the plant matures. The distribution ratio for stems, on the contrary, increases rapidly until early in July, then decreases with the advent of seed formation. In the later stages of development, a considerable part of the dry matter gain is directed to seed formation.

The most significant change which is caused by increasing the planting density is the increase in proportion of dry matter distributed to non-photosynthetic organs at the expense of that used for leaves. From the phytoecological point of view, it seems to us that the light and nutrient

salt conditions may be closely connected with these results, although we have, as yet, no information concerning the physiological side of this problem.

During the period from 18 June to 25 June 1955, the distribution ratio to leaves, stems, roots and reproductive organs was found to be 19, 67, 9 and 6 per cents, respectively, in the 5 cm. plot. If it is assumed that the additional dry matter is used immediately for the reproduction of each organ, the dry matter gain of 11.4 g. of 18 June may be expected to be distributed to each organ in the following way: 2.2 g. to leaves, 7.6 g. to stems, 1.0 g. to roots and 0.6 g. to reproductive organs. Consequently, the dry weight of leaves, stems, roots and reproductive organs on 19 June must become 43.7, 31.8, 6.1 and 0.6 g./sq. m., respectively (Table 5). In the similar

Table 5. Dry matter production and reproduction in 5 cm. plot on 18 June 1955.

	June 18 Dry weight g./sq.m.	Leaf area index (F)	Daily net assim. g.	Daily respiration g.	Daily net-product. g.
leaves	41.5	1.59	14.0	—	
stems	24.2	—	—	2.2	
roots	5.1	—	—	0.4	
total	70.8	1.59	14.0	2.6	11.4

	June 18 Dry weight g./sq.m.	Distribution ratio %	Daily growth g./sq.m.	June 19 Dry weight g./sq.m.
leaves	41.5	19	2.2	43.7
stems	24.2	67	7.6	31.8
roots	5.1	9	1.0	6.1
reproductive organs	—	5	0.6	0.6
tatal	70.8	100	11.4	82.2

way, the dry weight of each organ of 20 June 1955, can be estimated from the values of 19 June 1955. By repeating such calculations, the successive course of growth, from 18 June to 23 July 1955, can be constructed theorectically. The theoretical growth curves thus obtained are shown in Fig. 1 (broken line). A comparison of the calculated with the directly measured dry weight of each organ is also given in Table 6. From these results it can be said that the calculated dry weight of the whole plant corresponds fairly well with the observed. Strictly speaking, particularly in the earlier stages, the calculated values tended to be lower than the observed. But, as a whole, the real course of growth in each buckwheat stand may be said to accord well with the theoretical calculation of the production as well as the reproduction of dry matter.

Table 6. Comparison of the calculated values with the observed ones for dry weight of each organ. (g./sq.m.) (1955)

5 cm. plot	June 18		June 25		July 2		July 9		July 16		July 23	
	obs.	cal.	obs.	cal.	obs.	cal.	obs.	cal.	obs.	cal.	obs.	cal.
leaves	41.5	—	87.5	58.3	57.2	65.0	85.2	67.1	84.8	67.1	77.6	67.1
stems	24.2	—	118.4	81.6	147.2	125.2	264.0	188.4	306.0	211.2	345.6	235.4
roots	5.1	—	18.0	12.8	20.8	18.6	22.6	28.3	47.2	32.2	44.0	34.0
reproductive organs	—	—	6.8	4.1	14.4	7.5	46.8	48.4	108.0	102.2	154.8	153.4
total	70.8	—	230.7	156.8	239.6	216.3	418.0	333.2	546.0	412.5	622.0	489.9
10 cm. plot												
leaves	11.6	—	48.4	42.3	90.8	67.9	92.4	76.0	96.0	83.8	107.3	96.3
stems	5.1	—	44.0	37.4	165.2	110.6	225.1	193.2	237.9	211.9	254.2	221.2
roots	1.0	—	7.3	6.2	28.6	16.9	29.9	33.6	34.2	47.3	57.5	55.9
reproductive organs	—	—	2.5	2.1	14.8	9.6	32.6	42.4	88.6	111.7	125.4	218.4
total	17.7	—	102.2	88.0	299.4	205.0	380.0	345.2	456.7	454.7	544.4	591.8
20 cm plot												
leaves	3.8	—	27.3	24.1	63.7	58.6	61.2	58.5	58.9	58.5	76.3	58.5
stems	1.5	—	14.5	12.5	65.1	60.4	127.7	155.4	166.5	215.3	225.2	264.6
roots	0.4	—	2.9	2.6	13.3	13.3	37.2	48.3	23.2	78.3	98.6	110.4
reproductive organs	—	—	0.7	0.6	5.7	5.4	20.7	29.4	46.4	59.6	89.9	96.2
total	5.7	—	45.4	39.8	147.8	137.6	246.8	291.6	295.0	411.7	489.9	529.7

The agreement of the calculated values with the observed ones indicates that the three factors suggested above, i.e. the higher relative light intensity in the plant community, the higher rate of photosynthesis in the leaves and the lower value of the C/F ratio, may be the real causes of the higher values of NAR.

From the results of this comparison, it may be concluded that the growth curve of the community can be composed by the analytical-synthetical method, and it is also expected that even the final yield of economically important parts of field crops (seeds, fruits and roots, etc.) may be determined theoretically, when the light assimilation curve of the leaves, the rate of respiration of the plants and the distribution ratio of produced dry matter to the various organs are known and the main climatic factors, such as light and temperature, are measured quantitatively. It may moreover be expected that such a calculation will become more precise when the environmental factors in plant communities are investigated more precisely and the relationships between each of the important plant functions (photosynthesis, respiration, distribution of matter etc.) and the individual environmental factors are elucidated.

Summary

1. The influences of planting density on the dry matter production in a plant community were investigated, on the basis of the field experiments with buckwheat in 1954 and 1955.

2. Buckwheat plants were planted in regular square disposition and three different grade of spacing (5 cm., 10 cm. and 20 cm.) were employed. Sampling was made at intervals of 7 days for two months in each plot. The dry weights of leaves, stems, roots and reproductive organs and the total leaf area of the plants were measured.

3. Variations of the net assimilation rate (NAR) with time were determined for each buckwheat stand. From the results, it was indicated that the NAR diminishes with increasing density of stand, at least in the earlier stages of development (Fig. 3).

4. The maximum assimilation of buckwheat leaves in densely planted stands was found to be lower than that in stands with lower densities (Fig. 4).

5. The C/F ratio, the ratio of the non-photosynthetic systems (stems, roots and reproductive organs) to the photosynthetic system (leaves) was calculated for each plot. It was shown that the C/F ratio tends to become higher with increasing density in the earlier stages of growth, but in the later stages, no apparant correlation between C/F ratio and density was observed.

6. From the analytical consideration with regard to the dry matter production in buckwheat stands, it was concluded that the higher values of NAR of the widely spaced stands are mainly due to 1) the higher relative light intensity in the plant community, 2) the higher rate of photosynthesis of the leaves and 3) the lower value of the C/F ratio than that of the closely spaced stand.

7. The dry matter production of buckwheat plants was calculated indirectly from the daily assimilation and respiration, and the growth curve of the standing crop was constructed theoretically for each stand. The results of the calculation agreed well with those of the direct determination.

The agreement of these values indicates that the growth curve of a plant community can be composed indirectly by the calculation of dry matter production and reproduction in the community.

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Taxonomical Studies on the *Subsecunda* Group of the Genus *Sphagnum* in Japan, with Special Reference to Variation and Geographical Distribution.*

By

Hyoji Suzuki

I. Introduction

The *Subsecunda* group has been regarded taxonomically as the most difficult in the genus *Sphagnum*, because a slight ecological condition exerts variations or differences in habit and anatomical characters. This has led to divergent views and treatments of the species. Warnstorf (1911) reported 116 species of the group in the world. However, there are many bryologists who doubt the validity of the species of *Sphagnum* as treated by Warnstorf, and his works have been more or less criticized and revised. The numerous species described by him have been reduced as the result of subsequent revisions. On the other hand, 12 species of this group listed from Far Eastern Asia by Ishiba (1924, 1932) and 11 from Japan by Sakurai (1954) are based on the work of Warnstorf (1911).

The writer commenced his studies on this group in Japan with the purpose of throwing more light on the subject. The present paper is the conclusion reached after studying about 700 specimens from approximately 140 localities in various parts of Japan and from observations of the natural growing conditions.

II. Observation on the variation of *S. subobesum*

From the peculiarities of the members of the *Subsecunda* group (usually hydrophytes or hygrophytes), considerable variation can be observed even in a population of a small area. Here the writer deals with the results obtained from the investigation of various growing forms of the species collected at one locality on a low plateau in northeastern Honshu.

Materials were collected at two stations within a distance of about 500 m, one is a marshy place developed at the upper margin of a small water-reservoir and the other a small, more or less desiccated peat-bed. To make clear the tendencies and ranges of variations in external and

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structural characteristics of this moss, the writer classified the materials into five forms according to their conditions of growth, mainly caused by the intensity of light and degree of submergence: that is, dried, normal, shaded, emersed and submerged forms.

1. Variation in external habits.

The stem of this moss shows a tendency to be shortened in dry condition and to be elongated in water. This moss is usually deep reddish brown at the coma, especially in the cold season, and is discolored below and often stained near the base by rust brown water contained in the substrata. The shaded moss commonly is more green than brown. The water form of this moss, i.e. emersed or submerged form, like the water forms of most of the brown colored *Sphagna*, is tinged with peculiar dark purple. It is rather interesting that the dried specimen of brown color generally changes to similar dark purple when it is dried again after soaking.

The branch-fascicles are normally composed of four branches, two of which are spreading and two pendent. The number of branches generally decreases both in the dried and submerged forms, and there is a tendency of the branch-fascicles being arranged rather densely in the dried, and looser in the shaded and submerged forms. Spreading branches are normally slender, being 10–15 mm long, and become thicker and longer below. They are usually short in the dried form and become slender in the shaded one. On the other hand, they are much thicker and longer in the water forms. The branches of the normal form curve slightly downward, but comal branches of the dried and emersed forms usually show a strong curve in the shape of a circle or spiral. The latter characteristic is remarkable in the *Subsecunda* group, though it slightly varies according to the species and their growing conditions. Horizontal branches are often recognized in the water forms.

Branch-apex is usually acute or awl-shaped, often flagelliform in plants regenerated on dried peat or soil, and obtuse in most water forms.

Branch-leaves give the branch various appearances according to the degree of recurvation of leaf-apices. Comal branches are normally smooth, those in the middle part of the stem are more or less serrate, and those of the lower part smooth again. The dried form usually shows smooth branches, the shaded and emersed ones have more or less serrate branches while the submerged form has peculiar plumous branches with divergent or diffused leaves.

As mentioned above, the external habits of this group are so variable that many authors neglected them. However, some German workers established many varieties and forms based on these characteristics. The writer believes that these characteristics are of value when compared with each other, considering the tendencies and ranges of their variations.

2. Variations in anatomical structures.

As the important characteristics in anatomical structures of this group, the following may be taken into consideration: number of cortical layers of stem; shape, size, fibrillation, division and perforation of both stem- and branch-leaves.

Juvenile plants, which are often encountered in most species of *Sphagnum,* are important for the study of variations in anatomical structure. They are usually found in the innovation in various parts of the plants, for example, in apical, middle or basal parts of the branch, and on young or injured stems. Young stems arisen from innovation usually lack branches at the start and bear stem-leaves similar to normal branch-leaves. A single branch grows on the stem thereafter, and new branches grow at its base, then the branch-fascicles, which are characteristic to the typical peat-mosses, are accomplished. Åberg (1937) classified three stages of stem development in juvenile plants: *subsimplex,* lacking or having only few single branches; *monocladous,* bearing single branches; and *oligocladous,* bearing branch-fascicles composed of 2-3 branches. From the examination of a packet of 156 stems belonging to the dried form, the writer could divide them into 55 (35 per cent) subsimplex, 52 (34 per cent) monocladous, 36 (23 per cent) oligocladous and 13 (8 per cent) typical stems.* Such juvenile plants are also very frequently observed in the submerged form (Figs. 1.-A,-B) as in the dried and often in dense cushions of the normal form.

The most interesting point in the observation of these juvenile plants is in the variation of the stem-leaves. Åberg (1937) recognized the gradual differentiation of stem-leaves corresponding to stem development in juvenile and typical plants and classified them into the following five types according to their development:

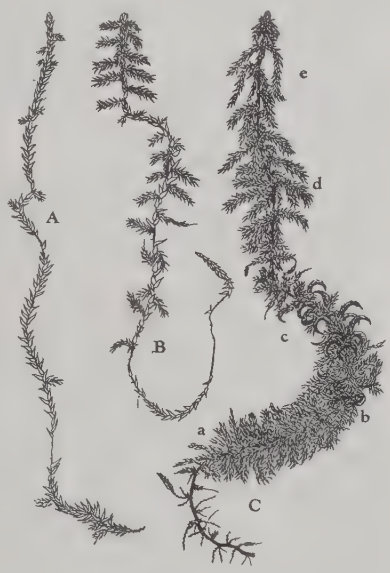


Fig. 1. Examples of modifications recognized in submerged plants of *S. subobesum* ×3/5.

A...Subsimplex, B...Monocladous, C ...Oligocladous, bearing smooth and strongly curved branches in the middle part of the stem (**c**), **a**~**e**--- indicate the parts used in the comparative study on important characteristics (cf. Tab. 2). (H.S. 1315).

* Typical stem (or plant) called by the writer in this article means the non-juvenile one.

Isophyllous type: stem-leaves almost entirely similar to branch-leaves,
Subisophyllous type: stem-leaves completely or almost completely reinforced with fibril-bands, differing slightly from branch-leaves by their shape and broader base,
Hemiisophyllous type: stem-leaves more or less resembling the branch-leaves in the upper part only, fibril-bands vanishing in the lower part and with somewhat broadened border near the base,
Subanisophyllous type: stem-leaves somewhat resembling the branch-leaves only in the fibril-bands in their apical parts, but differing more or less from the latter in perforation,
Anisophyllous type: stem-leaves almost entirely without fibril-bands, differing completely from the branch-leaves in perforation when they are perforated.

This classification is useful for the study of variations in this group. In Table 1 is shown the result of observations on the variation in the stem-leaves corresponding to the stages of stem development of the dried and submerged forms.

In the dried form, as can be seen from Table 1, stem-leaves of juvenile

Table 1. Variations in stem-leaves corresponding to the stages of stem development observed in the dried and submerged forms of *S. subobesum*.

A. Dried form

Stage of development: / Characters	subsimplex	monocladous	oligoclaedous	typical (polycladous)
Size (mm)				
Width at base	0.50—0.65	0.40—0.55	0.55—0.66	0.50—0.62
Width at widest part	1.33—1.45	0.79—0.83	0.83—0.95	0.70—0.83
Length	2.73—2.91	1.40—1.60	1.80—2.00	1.20—1.33
Shape	ovate oblong	round ovate	ovate oblong	ovate lingulate to triangular lingulate
Fibrillation	whole surface	whole surface	whole surface	upper 1/4 to whole surface
Perforation				
Inner surface	± numerous	numerous in middle part	±numerous	± numerous
Outer surface	numerous	numerous at upper region	numerous	numerous at upper 1/3
Comparison*	inn. < out.	inn. ≈ out.	inn. < out.	inn. > out.
Shape of apex	truncate with a few teeth	truncate with teeth or hyaline margin	narrow truncate with teeth or hyaline margin	truncate with hyaline margin

B. Submerged form.

Stage of development: / Characters	subsimplex a	subsimplex b	oligocladous a	oligocladous b
Size (mm)				
Width at base	0.20–0.30	0.37–0.58	0.50–0.54	0.50–0.70
Width at widest part	0.90–1.10	0.80–0.92	0.70–0.84	0.75–1.00
Length	2.10–2.30	1.70–2.00	1.60–1.80	1.40–1.60
Shape	elongated ovate	ovate	ovate	elongated ovate
Fibrillation	whole surface	whole surface	whole surface	whole surface
Perforation				
Inner surface	nearly absent	± numerous	not numerous	not numerou
Outer surface	nearly absent	not numerous	nearly absent	±numerous on upper half
Comparison*	inn. > out.	inn. > out.	inn. > out.	inn. ≈ out.
Shape of apex	round or obtuse, entire or minutely dentate, composed of narrow cells	acute or truncate with indistinct teeth	truncate with 3–5 teeth	truncate with 4–6 teeth

* The expression, inn.<out. shows that the pores are more numerous on the outer surface than on the inner surface of the leaves, inn.>out. vice versa, and inn.≈out. shows that the number of pores is approximately equal on both surfaces of the leaves.

plants are ovate oblong, being fibrillose almost down to the base and are generally larger than those of the typical stem, while those of the latter are ovate lingulate or triangular lingulate and are provided with fibrils on the upper quarter of the leaf or farther below and rarely down to the base. In the submerged form, they are ovate or elongated ovate, but show similar tendencies in their size and fibrillation to those of the dried form.

It is very interesting that perforations in the stem-leaves of juvenile plant show different tendencies between the dried and submerged forms. In the dried form, the pores of the stem-leaves are more numerous on the outer surface than on the inner as in the normal branch-leaf, while in the submerged form they are very few on the whole and seemingly show a reverse condition. From these phenomena we may say that the stem-leaves in the dried form are still in a primitive stage of differentiation, while those in the submerged form show degeneration by diminution of pores due to the submerged habitat. Moreover, there are some peculiar cases where the number of pores are approximately equal on both surfaces of the leaf in both dried and submerged forms. It is important to notice such ambiguous cases occasionally encountered in abnormal conditions, whenever we try to compare the number of pores on the inner surface with that of the outer. Indeed, most ambiguos forms which sometimes occur and are difficult to identify are recognized as those grown in unfavorable conditions.

It is clear from the table that various gradations of development in the apices of the stem-leaves according to the stem development are observed in both dried and submerged forms, but peculiar apices composed of only narrow cells as in the border, are often observed in the stem-leaves of the submerged form, especially in extraordinarily enlarged leaves of subsimplex stems.

The writer found several oligocladous individuals in the submerged form bearing smooth and strongly curved branches in the middle part of the stem (Fig. 1.-C). From these strongly curved branches, it may be conjectured that these plants have experienced an emersed condition in the course of development. A comparison of the important characteristics

Table 2. Variations of some important characters in various parts of an individual of *S. subobesum* bearing strongly curved branches in its middle part. (**a** ...lowest part, **b** ...part lower than curved branch region, **c** ...curved branch region, **d** ...part above the curved branch region, **e**...part below the head, **f**...typical plant of the normal form.)

Parts on stem / Characters	a	b	c	d	e	f
Cortical layers	(1)−2	(1)−2	(1)−2	(1)−2	(1)−2	(1)−2
Ratio of 2 layered part to periphery	1/2−3/4	1/2−3/4	more than 3/4	less than 1/4	less than 1/4	more than 3/4
Composition of branch-fascicle*	2A+H (2A+O)	2A+0 (2A+H)	2A+0 (2A+H)	2A+0 (A+H)	2A+H	2A+2H (2A+H)
Stem-leaf						
Shape	elongated ovate lingulate	ovate	ovate to ovate linguate	triangular lingulate	ovate	triangular lingulate to ovate lingulate
Size (mm)						
Length	2.20−2.50	1.80−2.40	1.70−2.01	1.40−1.50	1.20−1.40	1.00−1.47
Width	0.60−0.80	0.80−0.90	0.63−0.84	0.84−0.92	0.71−1.00	0.67−0.92
Fibrillation	3/3 (subiso.)	3/3 (subiso)	upper 2/3 (hemiiso.)	upper 2/3 (hemiiso.)	upper 1/2 (hemiiso.)	upper 1/3 (subaniso.)
Apex	±obtuse, composed of narrow cells	damaged or obtuse with some teeth	obtuse with ca 10 teeth, often cucullate	truncate with hyaline margin on both sides	truncate with ca 5 teeth and hyaline margin, often cucullate	roundly truncate with hyaline margin, often cucullate

* 2A+H shows that the branch-fascicles are mainly composed of two spreading and one pendent branches and (2A+0) shows that those of only two spreading branches with no pendent one are occasionally observed.

Pores per cell						
Inn. surface	6	8	5	6	5	more than 8
Out. surface	0—1	0—1	0—1	0—1	1—2	1—2
Comparison	inn. > out.	inn. > out	inn. > out.	inn. > out.	inn. > out.	inn. > out.
Branch-leaf						
Shape	ovate lanceolate	elongated ovate	elongated ovate	ovate lanceolate	ovate oblong	ovate lanceolate to round ovate
Size (mm)						
Length	3.50—3.70	4.00—4.40	3.00—3.60	2.40—2.70	4.20—4.26	1.50—2.40
Width	1.20—1.50	1.70—1.80	1.20—1.50	0.75—0.85	1.40—1.55	0.50—1.00
Apex	acute or truncate with 3—8 teeth	narrow truncate with 6—8 teeth	narrow truncate with teeth	narrow truncate with 5 teeth	narrow truncate with 5 teeth	narrow truncate with 7—8 teeth
Pores per cell						
Inner surface	0—2	2—3	0—2	2	2—4	1—2
Outer surface	2	± numerous	± numerous	0—4	± numerous	numerous
Comparison	inn. < out.	inn. < out.	inn. < out.	inn. ≈ out.	inn. < out.	inn. < out.

of the various parts in one of such individuals were made and the result is shown in Table 2 with the observation on a representative of typical plants of the normal form.

Many bryologists believe that the cortical layers of the stem are comparatively stable, so that their number serves to distinguish the species or higher units of this group. Although the cortical part in these plants is composed of one to two layers, it is evident that the ratio of the two-layered parts in the circumference shows large fluctuation even in the same stem, as shown in Table 2. Similar fluctuation, to some degree, is also observed in the number of branches in a branch-fascicle.

The stem-leaves of the typical plants are normally triangular lingulate to ovate lingulate, and their apices are roundly truncate and often somewhat cucullate with hyaline margin. When they develop sufficiently, they usually have fibrilbands only in the area of slightly less than one third of the upper part of the leaf (subanisophyllous). As seen in Table 2, however, stem-leaves of the oligocladous plants are generally larger, more rounded and more fibrillose than those of the typical ones, but exceptionally triangular lingulate leaves were observed in part **d** (part above the curved branch region). As to the degree of fibrillation, successive changes were observed from subisophylly to hemiisophylly toward the stem-apex. The structure of their apices also successively changes toward the stem-apex; that is, the apex of the leaf in the lowest part is composed of narrow cells, that in the above two parts is denticulate with chlorophyll-cells and that in the uppermost two parts has hyaline margins on both sides of the truncated apex, as commonly observed in the typical plants.

The branch-leaves of the oligocladous plants are larger, flatter and more elongated than those of the typical ones. No remarkable structure

in the apices of the branch-leaves was observed in our plant, but some extraordinarily enlarged leaves (4.24–4.66 mm long and 1.68–2.18 mm wide) with apices composed of only narrow cells were detected in other individuals of this form. It is interesting that the perforation of the branch-leaves is highly variable and corresponds to the change of environmental conditions, that is, the branch-leaves in both the lowest part (**a**) and in part (**d**) just above the curved branch region (**c**) have only a few pores per cell, while those in the remaining parts have fairly numerous pores on the outer surface like those in the typical plants of the normal form.

Compared with the great variation recognized in both juvenile and oligocladous submerged plants, no remarkable differences in important characteristics for distinguishing taxonomic units could be found among the five forms classified by their external habits and their growing conditions.

Moreover, in connection with the perforation of the branch-leaves, there are common characteristics among these five forms. In other words, they coincide with one another in having the following characteristics; the branch-leaves usually have none or few pores on the inner surface, and in the latter case, the pores are small, round, ringed or ringless and situated at the lower end or at both ends of the cells, besides, there are some pseudopores or pseudopore-rudiments at the corners of the cells and often in short rows along the commissures, especially at the apical region; the outer surface generally has more or less numerous pores which are arranged in discontinuous rows along the commissures always intermixed with pseudopores or pseudopore-rudiments.

From these facts, it is clear that the five forms mentioned above are not independent units or species, but only modifications induced by the difference of environmental conditions.

The writer believes that such tendencies or ranges in variations of the important characteristics should be taken into consideration in studying this group.

III. Classification and Discussion

During the course of investigation on the variation on this group in each locality, the writer confronted with several packets collected from different localities in Japan including two or three members which belong to the *Subsecunda* group and which are distinguishable from one another even by their external habits and anatomical structure.

For example, three different members as shown in Fig. 2 and Table 3 were recognized in a packet (H.S. 21225) collected at the margin of a small depression on a peat-bed covering the lower margin of a lake near Toyokoro Railway Station, Prov. Tokachi in Hokkaido. These members represent independent species, because they show different characteris-

tics even under the same circumstances. These members are to be referred respectively to *S. subsecundum, S. kushiroense*,* and *S. subobesum.* The most important characteristics for distinguishing these species are

Fig. 2. Three different members recognized in a packet (H.S. 21225). S...*S. subsecudum*, K...*S. kushiroense*, O...*S. subobesum*. Upper...Stem-leaves ×23, Middle...Branch-leaves ×23, Lower...Perforations in hyaline cells of the middle part on the outer surface of branch-leaves ×335.

to be found in the branch-leaf, especially in the perforation on the outer surface; i. e. large, ringed or bordered pores arranged in continuous rows along the commissures in *S. subsecundum,* weakly ringed or ringless pores in rather discontinuous rows in *S. kushiroense,* and small strongly ringed or bordered pores in discontinuous rows intermixed with some pseudopores

* This is described in this paper as a new species closely allied to *S. microporum.*

Table 3. Comparison of external and anatomical chracters of three different members recognized in a packet (H. S. 21225). (N. B. The data in this Table do not always show the typical characters of each species.)

Members: / Characters	*S. kushiroense*	*S. subsecundum*	*S. subobesum*
External character			
Coloration	pale or yellowish green to brownish yellow	pale green to yellowish brown	yellowish green with purple
Habit	delicate (8 cm) soft	delicate (7–8 cm) soft	robust (5–7 cm) rigid
Cortical layers	1	1–(2)	1–2 (inner layer with narrow lumen)
Composition of branch-fascicles	2A+3H (2A+2H)	2A+3H (3A+3H) (3A+4H)	3A+3H (3A+6H)
Aspect of comal branches	serrate	smooth	smooth
Stem leaf			
Shape	triangular lingulate	triangular lingulate	lingulate
Size (mm)	0.90–1.05×0.52–0.60	0.97–1.01×0.60–0.75	1.27–1.45×0.52–0.56
Fibrillation	none or only at apex	none or only at apex	upper 1/2 to 3/5
Divisions	numerous, often double	rare	very rare
Pores			
Inner surface	numerous ringless pores near apex	numerous large ringless pores in upper region	ringed and ringless pores along the commissure, large ringed or bordered pores at corners of cell
Outer surface	none or few ringless endpores or free pores near apex	none or few endpores in apical region	ringed or bordered pores in short rows in apical region
Branch-leaf			
Shape	ovate lanceolate, strongly falcate	ovate oblong, slightly falcate	ovate, scarcely falcate
Size (mm)	1.68–1.80×0.78–0.90	1.31–1.50×0.75–0.83	1.87–2.06×0.93–1.05
Apex	acute to acuminate	obtuse or mucronate	acuminate
Upper margin	strongly involute	weakly involute	slightly involute
Size of hyaline cell (μ)			
Upper	56–72×9–12	50–63×9–12	107–116×12–15
Middle	116–148×11–16	94–113×12	163–182×15–18
Basal	157–179×14–16	119–148×17–25	195–221×15–23
Pores (outer surf.)			
Kind	weakly ringed or ringless, in discontinuous rows	ringed or bordered, in continuous rows	strongly ringed or bordered, in discontinuous rows, always intermixed with some pseudopores or pseudopore-rudiments

Size of opening			
Diameter in μ	3.7—5.6 (various)	4.7—5.3 (large)	3.1—4.4 (very small)
Ratio to width of hyaline cell	2.85%	40.0%	13.3%

or pseudopore-rudiments in *S. subobesum.* The nature of the stem-leaves are also useful for distinguishing the species; that is, subanisophyllous stem-leaves with large pores on the inner surface and numerous, often double or more complicated divisions in hyaline cells are the characteristics of *S. kushiroense,* and hemiiso- to subanisophyllous stem-leaves with large, ringed or bordered pores at the corner of hyaline cells on the inner surface are the features of *S. subobesum.*

When Åberg (1937) classified this group in Europe into three species, i. e. *S. pylaiei, S. contortum* and *S. subsecundum,* he stated that the first two species are characteristic in having two or numerous layers of cortical cells of stem and the last as having only one layer; then he classified, mainly by the degree of isophylly of stem-leaves, the second into two and the last into five varieties.* However, the degree of isophylly of the stem-leaves does not appear to be always reliable for the classification of this group, because it is not only quite variable but also anisophyllous stem-leaves are often encountered in different members, as shown in Table 3. Greater emphasis should be given to the nature of pores in the stem- and branch-leaves, as far as the Japanese species are concerned. The writer believes that a combination of the following characteristics may bring about a more natural classification of this group; that is, external appearance, number of cortical layers, perforations and divisions of stem-leaves and perforations of branch-leaves, especially the nature, number and distribution of the pores on both surfaces.

From the investigation on the Japanese materials belonging to this group, the writer recognized the following eight units, as shown in the following key, if the variations of each feature are to be considered. These units classified by the writer's own principle, have more or less different ranges of distribution in Japan, as is clear from Map 1 and Fig. 4. Thus, the writer favors to deal with these units as species rather than varieties.

Key to the Japanese species

1. Pores of branch-leaves large enough to join together, arranged in continuous rows along the commissures, numerous on both surfaces or only on the outer..... 2
   Pores of branch-leaves rather too small to join together, arranged in rather discontinuous rows along the commissures, more numerous on the outer surface than on the inner..... 5
2. Pores of branch-leaves numerous on both surfaces..... 3
   Pores of branch-leaves numerous only on the outer surface..... 4
3. Plants with normal branch-fascicles; stem-leaves anisophyllous, small, triangular

* Åberg's varieties roughly correspond to the writer's species.

lingulate; po.es on the outer surface of cortical cells indistict .....
.....1. *S. calymmatophyllum*
Plants subsimplex; stem-leaves isophyllous, large, ovate lingulate; pores on the outer surface of cortical cells distinct .....2. *S. guwassanense*
4. Cortical cells of stem in 1 (–2) layers .....3. *S. subsecuncum*
Cortical cells of stem in (1–) 2 (–3) layers.....4. *S. contortum*
5. Outer surface branch-leaves with ringless or weakly ringed pores and always without pseudopores or pseudopore-rudiments; stem-leaves commonly with numerous, often compound divisions..... 6
Outer surface of branch-leaves with bordered pores with strong rings, always with pseudopores or pseudopore-rudiments; divisions rather rare in stem-leaves 7
6. Stem-leaves usually hemiisophyllous, with small pores on the inner surface; capsules 1.3–1.5 mm in diam. and spores small (20–25 μ in diam.).....5 *S. microporum*
Stem-leaves usually anisophyllous, with large pores on the inner surface; capsules 1.87–1.95 mm in diam. and spores large (30–32 μ in diam.).....6. *S. kushiroense*
7. Stem-leaves with numerous pores on the inner surface; cortical cells of stem in 1–2 layers .....7. *S. subobesum*
Stem-leaves with few pores (generally endpores only) on the inner surface; cortical cells of stem in 2 (–3) layers.....8. *S. platyphyllum*

Reference should be given to the taxonomical value of the various stages of development recognized in the stem-leaves. The occurrence of stem-leaf types and their variation in length observed in the respective species classified by the writer are graphically shown in Fig. 3, excepting *S. guwassanense* and *S. platyphyllum*. The smooth curves are drawn based on the range* in length of stem-leaves in each specimen observed. When a specimen has two or more types, it was classified as belonging to the type of the lowest stages of development. As seen in the figure, the dominant types are different between the upper four species and the remainders. It may, therefore, seem adequate to classify the species or other units by means of stem-leaf types, as done by some bryologists, but it seems more reasonable to recognize the fact that various types of stem-leaf may occur even in the same species corresponding to the degree of development caused by the growing conditions. On the other hand, the length of the stem-leaves generally increases from the anisophyllous type to the subisophyllus type in each species; especially this is evident in *S. microporum* and *S. subobesum*. But the hemiiso- or subisophyllous stem-leaves of *S. microporum* are unexpectedly short. In other words, the length of the stem-leaves of this species is rather stable. The writhr, however, believes that the types and size of stem-leaves are not always helpful to distinguish the taxonomical units, if other characteristics are not taken into consideration.

* The writer has drawn the figures assuming that all the leaves belonging to all classes occur within a certain range, because he has observed that variation in length of 675 stem-leaves of *S. junghuhnianum* ssp. *pseudomolle* shows a normal distribution (Suzuki, 1956). Observed numbers of stem-leaves are 20, 25, 93, 18, 218 and 342 respectively, from the top species to the bassal ones in the figure.

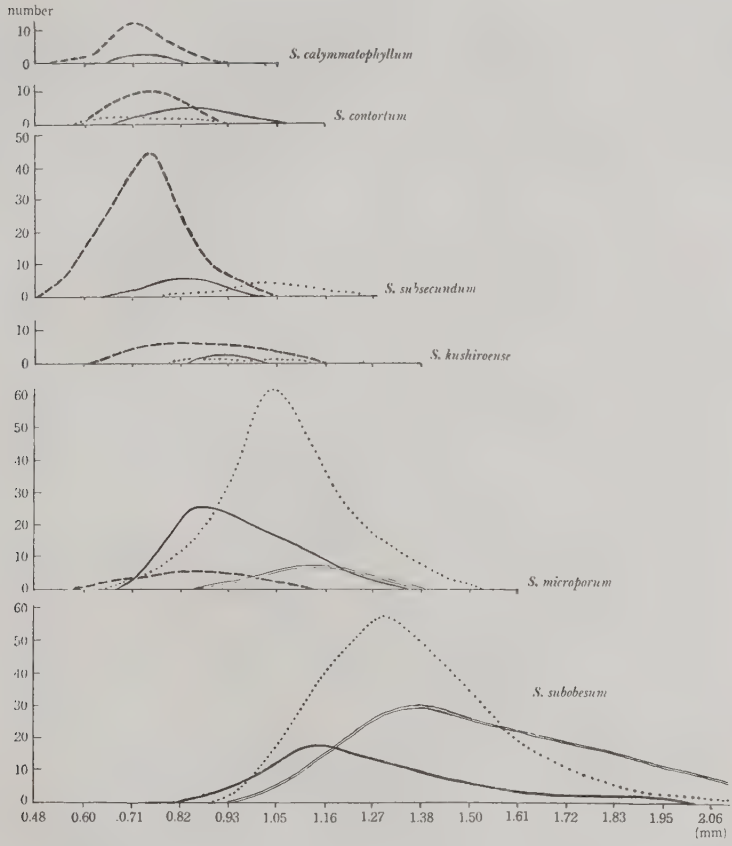
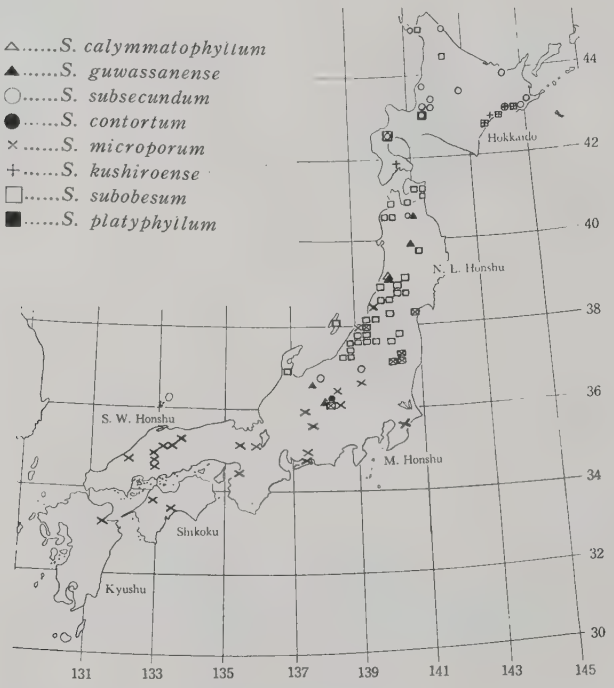


Fig. 3. Occurrence of stem-leaf types and their variations in length in the species of the *Subsecunda* group in Japan.
- - - -...Aniso- ——...Subaniso- .....Hemiiso-
=====...Subisophyllous leaves

Further details of the species given in the key are presented below to make clear their inter-relationship.

1. *S. calymmatophllum* and *S. guwassanense* including its three ssp. *guwassanense, takedae* and *triseriporum* have a common characteristic in bearing numerous pores on both surfaces of branch-leaf, and hold a special position among the members of *Subsecunda* group in Japan. It is seemingly true that *S. guwassanense* may be differentiated from *S. calymmatophyllum*, judging from the following interesting facts: *S. calymmatophyllum* grows

near the summit of Mt. Gassan, while *S. guwassanense* ssp. *guwassanense* grows luxuriantly on the slopes of this mountain being situated nearly in the center of the range of *S. guwassanense* (*sensu lato*) and some stem-leaves of juvenile plants of *S. calymmatophyllum* closely resemble those of the well developed forms of ssp. *triseriporum*, excepting that the latter usually has more numerous free ringless pores. However, the writer treated these



Map. 1. Geographical distribution of members of the *Subsecunda* group in Japan.

two as independent species, because *S. calymmatophyllum* is distinguishable from *S. guwassanense* not only by the characteristic mentioned in the key, but also in that the former has no kind of pseudopores that are commonly observed on the inner surface of the stem-leaves and also of branch-leaves, if any, in the members of the latter.

2. *S. subsecundum* and *S. contortum* are easily distinguishable from the other species of the group by the perforation of their branch-leaves. They usually have, only on the outer surface of branch-leaves, large, numerous ringed or bordered pores arranged densely along the commissures like a string of beads. The pores are so large and so continuous that, when

stained, they make clearly broad hyaline frames around the chlorophyll-cells. Concerning the perforation of the branch-leaves, there are no differences between *S. subsecundum* and *S. contortum* in our material. But, all the specimens contained in 24 packets collected on Mt. Kirigamine, which apparently seem to belong to *S. subsecundum,* have dominantly two cortical layers in the stem and they show more robust habit and more numerous divisions in the stem-leaves than *S. subsecundum.* The writer, therefore,

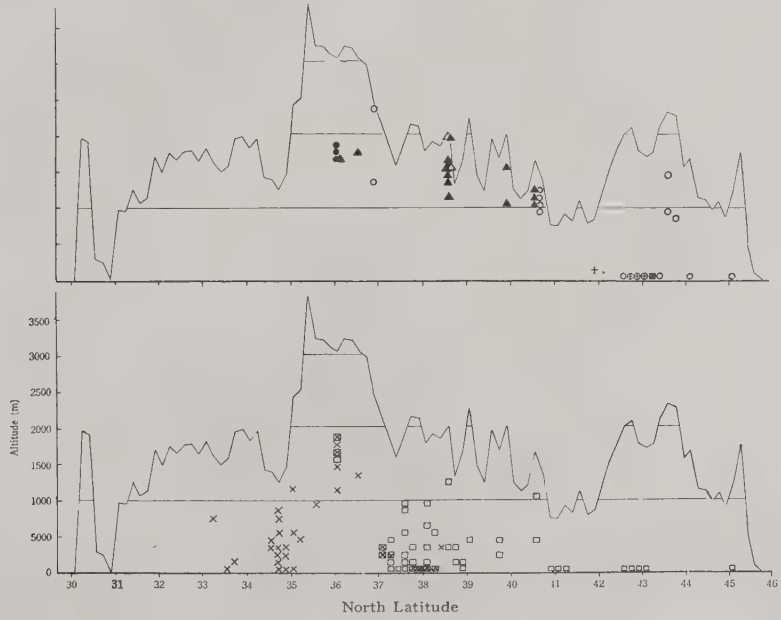


Fig. 4. Altitudinal and latitudinal distribution of the members of the *Subsecunda* group in Japan. Symbols are the same as in Map 1.

recognized these specimens as *S. contortum.* But, there is a question whether the Japanese materials strictly coincide with both species of European authors respectively, because the aspects of the perforations shown in the figures by Åberg (1937) are fairly different from those of the Japanese materials. However, the writer regards, at present, that these plants may belong to a certain form or forms of the European *subsecundum* or *contortum,* because he recognized a European specimen of *S. subsecundum* exhibiting similar aspects to the Japanese plants, and also a form of *S. contortum* bearing large pores has once been reported as its var. *reinkei* from Europe.

In connection with *S. subsecundum,* we must inquire into validity of *S. miyabeanum.* This species was established by Warnstof (1911) based

on a specimen, Miyabe no. 6, (July 27, 1884, Prov. Kushiro in Hokkaido) which was formerly considered by him as belonging to *S. subsecundum* (Brotherus, 1899). He said that the key characteristics distinguishing the two species were the smaller stem-leaves and the ringed pores, if present, on the inner surface of them in *S. miyabeanum*, and that the pores on the outer surface of its branch-leaves were less numerous than in *S. subsecundum*. The peculiality in these characteristics, however, are almost negligible when great variations of the respective characteristics in *S. subsecundum* are taken into consideration. The writer believes, therefore, that *S. miyabeanum* is merely an oligoporous form of *S. subsecundum* which is often encountered in the southeastern coastal regions of Hokkaido (i. e. in the provinces Kushiro, Tokachi and Iburi). It should be mentioned that a form bearing both strongly oligoporous branch-leaves (*miyabeanum*-type) and stem-leaves with ringless pores on the inner surface (*subsecundum*-type) was observed in Sizukari moor, Prov. Iburi. Moreover, there are various intermediate types between this and the normal form of *S. subsecundum*.

3. *S. microporum* and *S. subobesum* are widely distributed in Japan. It seems that the former corresponds to a form of *S. gravetii* (including *S. auriculatum* and *S. rufescens*) and the latter to that of *S. inundatum* (incl. *S. bavaricum*), judging from the description of the respective species by Åberg (1937) or Savicz (1954). However, for the present, the writer regards the Japanese species as independent ones, because he can find some distinctions between them, not only from the descriptions but also from his examination of some European specimens.

In the writer's collection, most of the materials from the southern parts of Japan approximately coincide with the original description of *S. okamurae* while the majority of the specimens from the northern parts of Japan belong to *S. subobesum*. On the other hand, the writer has reached the conclusion that *S. microporum* and *S. okamurae* are conspecific, as the result of consulting isotypes of both species and from an investigation on the variations of the latter. According to the original descriptions, the cortical part of the stem is one-layered in *S. microporum* while it is one- or, sporadically two-layered in *S. okamurae*; and divisions of the hyaline cells in the stem-leaves are rare in the former while they are numerous and often double or more complicated in the latter. However, the number of cortical layers does not serve to distinguish these two species, because authentic specimens of *S. okamurae* show frequently one-layered cortical cells. Although divisions of the hyaline cells of the stem-leaves are less remarkable in the isotype specimen of *S. microporum*, they were recognized in all the 25 stem-leaves observed and moreover were doubled in 14 (56 per cent) leaves out of them. Without doubt the isotype specimen of *S. microporum* should be included in a wide variation of *S. okamurae*. However, as the former name (1904) precedes the latter (1907), the Japanese plants hitherto known as *S. okamurae* should be called

S. microporum.

Moreover, several specimens which should be treated as *S. oligoporum* were collected in a community of *Phragmites vulgaris* in a locality in northeast Honshu. In the same locality, more or less typical forms of *S. microporum* were growing together, and these plants were connected with each other through intermediate forms. Therefore, the writer believes that *S. oligoporum* is merely an oligoporous form of *S. microporum*.

4. Plants closely allied to but differing from *S. microporum* in size of capsule and spore were observed at several stations in the southern coastal districts of Hokkaido. It is expected that these plants belong to only a polyploid form of *S. microporum,* judging from the report that the polyploid *Sphagnum* species generally have larger spore mother cells (Sorsa, 1956). However, the writer proposes a new species, *S. kushiroense,* based upon these plants, because they are distinguishable from *S. microporum* even by their anisophyllous stem-leaves.

5. From the examination of some authentic specimens, the following results were obtained ; that is, the isotype specimen of *S. usenense* is merely a form of *S. subobesum* which grows in shaded places, and the majority of the Japanese plants named *S. inundatum* belongs to *S. subobesum* and a part of them belongs to *S. microporum*. The details are as follows. Warnstorf (1911) cited as *S. inundatum* two Japanese specimens, i.e, Faurie's no. 3 from Aomori and no. 22 from Junsai-numa. Faurie's no. 3 and seven other specimens named *S. inundatum* (nos. 77 from Mt. Asama, 101 of Asamushi, 102 and 187 of Junsai-numa, 104 of Nayoro, 200 of Furumogo and 219 of Mt. Ontake) are kept in the herbarium of Kyoyto University. The specimen no. 3 shows a peculiar structure in bearing numerous pores on the inner surface of the branch-leaves, but the writer regards it, at present, as a multiporous form of *S. microporum*. Among the specimens cited above, two specimens from middle Honshu, nos. 77 and 219, belong to *S. microporum* and all the remainders are to be treated as *S. subobesum*.

Warnstorf (1911) described *S. microporum* var. *junsaiense* based on Faurie's collections at Junsai-numa in Hokkaido and designated two specimens (Faurie, nos. 56 and 58) in the original description. Faurie's collections numbered 56 and 58 are preserved in the herbarium of Kyoto University. But, the label of the former specimen bears the name *S. subobesum* and of the latter *S. imbricatum* var. *affine*. Moreover, these specimens were collected in Aomori and not in Hokkaido. Therefore, these specimens cannot be treated as isotypes. But, judging from the original description to the effect that the branch-leaves often bear short rows of pseudopores in the apical region of the inner surface, we may safely conjecture that this variety belongs to *S. subobesum*.

6. The most reliable report on *S. rufescens* in Japan is the citation by Warnstorf (1911): "Japan selten Faurie, n. 57, 77!" Faurie's collection numbered 57 is not found in the herbarium of Kyoto University, though this number is also cited under *S. subobesum* in his work. The specimen numbered

77 has been preserved in the herbarium under the name of *S. inundatum*, and it belongs to *S. microporum* as it has been mentioned above. Judging from such circumstances, it is possible that the specimens that had been identified as *S. rufescens* were renamed during the course of reexamination by Warnstorf, and therefore the occurrence of *S. rufescens* in Japan is very doubtful.

7. Finally a specimen which was reported by the writer (1955) as *S. platyphyllum* has numerous pseudopores on the inner surface of the branch-leaf and resembles a form of *S. subobesum* bearing numerous pseudopores. But, it is so different from the latter, not only in its external habit but also in the structure of both stem and stem-leaves, that the writer has regarded this specimen as a form of *S. platyphyllum*.

IV. Systematic description and geographical distribution

1. Sphagnum calymmatophyllum Warnstorf et Cardot ex Cardot, Bull. Herb. Boiss. Ser. 2. **7**: 711. (1907); Warnstorf, Hedwigia **47**: 97. (1907) & Pflanzenr. **51**: 391. *f.* 43 E. (1911). (Fig. 5)

Sphagnum ovalifolium var. *japonicum* Warnstorf, l. c. *pro syn.*

Plant rather small, (1–) 3–11 cm high, normally slender, erect, often decumbent with short stem, pale green to yellowish brown or often purplish brown at the coma and discolored in lower part, somewhat rigid when dry.

Wood-cylinder yellowish brown to reddish brown, of 2–4 layers of cells with strongly thickened walls; cortical cells of the stem in 1–2 layers, occasionally in 3 layers in some parts of periphery, rather small, on the surface quadrilateral, often with a large indistinct pore or thinning at the upper end of cell.

Stem-leaves small, short triangular lingulate, rarely somewhat ovate lingulate, (0.50–)0.60–0.80(–0.90) mm long and (0.32–)0.40–0.60(–0.67) mm wide at the base, commonly cucullate and denticulate at the apex, often somewhat praemorse, the border narrow, of 2–4 rows of narrow cells with pitted walls, not or slightly broadened toward the base, in the upper half often indistinct or somewhat fimbriate; auricles rather small. Hyaline cells rhomboidal near the apex, narrowing toward the base, often with simple divisions in cells of apical and lower side regions, commonly without fibrils, often with a few fibrils in the apical part of the leaf; on the inner surface with numerous large, ringless, round or irregularly formed pores along the commissures, often producing pseudofibrils, diminishing and irregularly formed toward the base, at the middle basal part with 1–2 large ringless pores near the upper end or corners of the cell; on the outer surface with less numerous pores than on the inner surface, in the apical part usually with ringless or rarely half-ringed, round or elliptic pores along the commissures, diminishing toward the base where there are a few end- or corner-pores.

Branches in fascicles of 4–5, rarely 2 or 6, 2 or 3 of which spreading,

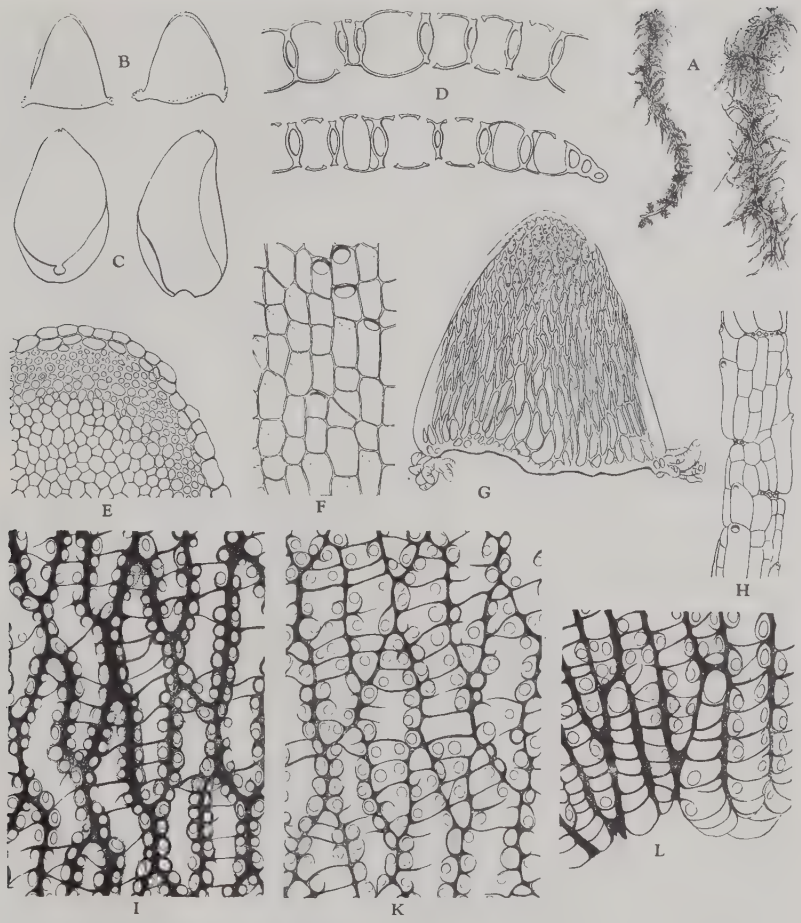


Fig. 5. *Sphagnum calymmatophyllum* Warnst. et Card. ex Cardot.
A. Sterile plants (1. ... H. S. 3023, r: ... H. S. 3041) ×3/4, B. Stem-leaves ×18, C. Branch-leaves ×18, D. Cross-sections of branch-leaf ×335, E. Cross-section of stem ×110, F. Outer surface of stem ×110, G. Areolation of stem-leaf ×58, H. Part of denuded branch ×58. I. Outer surface of the central portion of branch-leaf ×335, K. Inner surface of ditto ×335, L. Outer surface of the basal part of ditto ×335. (F. L. ... H. S. 3028, the others ... H. S. 3023).

up to 10 mm long, generally attenuate at the apex, their cortical cells in a layer, retort-cells with a pore and with rather conspicuous neck, usually 2 in a row. Branch-leaves ovate to round-ovate, strongly concave, often slightly bent to one side, 0.93–1.38 mm long and 0.56–0.90 mm wide, with

3–5 minute teeth across the narrowly truncate apex; the margin strongly involute, the border entire, narrow, of 1–2 rows of narrow cells. Hyaline cells short vermiform, with numerous fibrils and pores on both surfaces; on the outer surface with numerous pores, weakly or strongly ringed or bordered, arranged continuously along the commissures, the pores usually large and rounded, often as large as the cell-width near the upper ends of basal cells, besides, often with small, round, free pores on the median line of the cells; on the inner surface the pores similar in shape and arrangement to those on the outer surface, but more weakly ringed and less numerous in the lower part. The leaves of pendent-branches ovate to ovate lanceolate, with numerous larger pores, remembering the branch-leaves of the species belonging to *Palustria-* or *Acutifolia*-group.

Chlorophyll-cells in section of barrel-shape to flask-shape, the lumen fusiform, exposed on the outer surface or on both surfaces with thick walls, often almost enclosed on both surfaces.

Sexual organs or sporophytes are unknown at the present day.

Nom. Jap.: *Kobano-mizugoke.*

Distribution: Endemic to Japan (Honshu).

Specim. exam.: **N. E. Honshu***, Prov. Uzen, *Gassan* Higashitagawa-gun, Mt. Gassan, Midagahara, 1510 m (H. S. Aug. 24, 1946–H. S. 2961 + *S. guwassanense*,) ibid. near summit, 1970 m (H.S. Aug. 24, 1946–H.S. 3019 + *S. guwassanense*, 3020 + *S. guwassanense*, 3021 + *S. guwassanense*, 3022–3025, 3027 + *S. guwassanense*, 3028–3030, 3038 + *S. guwassanense*, 3041).

It is a very interesting fact from the standpoint of distribution that *S. calymmatophyllum* is restricted only to Mt. Gassan being situated almost at the center of the range of *S. guwassanense*. It grows most abundantly around a pond located near the summit, where it makes a moss carpet associating with *S. guwassanense*, *S. compactum* and a few phanerogamous plants, e. g. *Sanguisorba albiflora* and *Juncus* sp. In Midagahara it is observed in small amount intermixed with *S. guwassanense*.

2. **Sphagnum guwassanense** Warnstorf, Pflanzenr. **51**: 424. *f.* 74 E. 1911).

Sphagnum takedae Sh. Okam. in Matsum. Ic. Pl. Koishik. **3**: 109. *pl.* 200. (1917).

Nom. Jap.: *Gassan-mizugoke* (*sensu lato*).

Distribution: Endemic to Japan (Honshu).

The writer divided this species into the following three subspecies.

2a. Shagnum guwassanense Warnst. ssp. **guwassanense** H. Suzuki, Journ. Sci. Hiroshima Univ. Ser. B. Div. 2, **6**: 290. *f.* 2. (1954).

Sphagnum guwassanense Warnstorf l. c.

Nom. Jap.: *Gassan-mizugoke* (*sensu stricto*).

Specim. exam.:** **N. E. Honshu,** Prov. Mutsu, *Hachimantai*, Ninohe-gun, Mt.

* Division of districts in Japan was followed according to Horikawa (Horikawa, 1955) and the geographical names printed in italics indicate the names of the topographical maps (1 : 50,000) which include the localities of the examined specimens.

** Details of the specimens cited in previous papers (Horikawa & Suzuki, 1954; Suzuki, 1954, 1955) are omitted and only the respective localities are given.

Hachimantai, Hachiman-numa, 1570 m; Prov. Rikuchu, *Hachimantai*, Katsuno-gun, Mt. Hachimantai, Ōyachi moor, 1090m; ibid. Akita-nanbu-numa, 1530m; Prov. Ugo, *Chôkai-zan*, Akumi-gun, Mt. Chôkai, 1400-1600m; Prov. Uzen, *Gassan*, Higashitagawa-gun, Mt. Gassan, Midagahara, 1420m (H.S. Aug. 23, 1948 H.S. 2883–2888); ibid. 1510m; ibid. near summit, 1970m; ibid. Shōzokuba, 1320m; ibid. Mt. Ubagatake, 1400m (T. Suzuki-no. 368, Aug. 8, 1954–H.S. 19631), ibid. 1650m (T. Suzuki-no. 391, Aug. 8, 1954–H.S. 19635), ibid. Kiyokawagyōningoya, 1380 m (T. Suzuki-no. A45, July 23, 1955–H.S. 20369, -no. A73, July 25, 1955–H.S. 20372), ibid. Nenbutsugahara, 1200m (T. Suzuki-no. 482, Aug, 12, 1954–H.S. 19637).

2b. Sphagnum guwassanense Warnst. ssp. **takedae** (Sh. Okam.) H. Suzuki, Journ. Sci. Hiroshima Univ. Ser. B. Div. 2. **6**: 288. *f.* 1. (1954).

Sphagnum takedae Sh. Okam. l. c.

Nom. Jap.: *Ito-mizugoke*.

Specim. Exam.: **M. Honshu**, Prov. Shinano, *Suwa*, Suwa-gun, Mt. Kirigamine, Yashimagahara moor, 1650m (Y. Kubota-no. 296, July 1, 1950–H.S. 20385, -no. 399, July 3, 1950–H.S. 20392, T. Seki-no. 6632, Aug. 15, 1956–H.S. 21659), ibid. Yashimaga-ike, 1650m (S. Nakanishi, July 23, 1953–H.S. 17117, 17128), ibid. Ko-ike, 1650m; ibid. Kamaga-ike, 1650m; ibid. western part of the moor, 1640m (H.S. July 26, 1954–H.S. 18130, 18132 18152).

2c. Sphagnum guwassanense Warnst. ssp. **triseriporum** H. Suzuki, Journ. Sci. Hiroshima Univ. Ser. B. Div. 2. **6**: 294. *f.* 3. (1954).

Nom. Jap.: *Mitsuana-mizugoke*.

Specim. exam.: **N. E. Honshu**, Prov. Mutsu, *Hakkodasan*, Higashitsugaru-gun, Mt. Hakkoda, Senninta, 1300m; ibid. Shimokenashitai, 1050m; ibid. Kamikenashitai, 1150m; ibid. Yoko-numa, 1120m (H.S. Aug. 23, 1949–H.S. 7593, 7594), *Hachimantai*, Ninohe-gun, Mt. Hachimantai, Hachiman-numa, 1570m (H. S. Aug. 15, 1951–H. S. 12090, 12121, 12143); Prov. Ugo, *Hachimantai*, Senpoku-gun, Mt. Hachimantai, Seiun-numa, 1580m.

M. Honshu, Prov. Etchu, *Tateyama*, Nakaniikawa-gun, Mt. Tateyama, Midagahara, 1800m (H. Ando-no. 15779, Aug. 12, 1953–H.S. 17873).

Recently an example belonging to this subspecies was collected by Mr. H. Ando of our laboratory from Midagahara (1,800 m alt.) on Mt. Tateyama located in the central part of Honshu. Unfortunately the writer is not certain whether other subspecies grow on this mountain. In either case, the range of distribution of this species is exclusively confined at present to the northern half of Honshu on the Japan Sea, and the localities are situated at elevations of more than 1,000 m above sea-level.

It is a most interesting fact that *S. orientale* which was recently described by Savicz (1951) from the Siberian material is closely related to *S. guwassanense*, especially to ssp. *triseriporum* and that the latter not only has its range in the northern half of Honshu, particularly in the region on the Japan sea, but also it grows mainly on higher mountains.

3. Sphagnum subsecundum Nees in Sturm, Deutch. Fl. Crypt. II. 17. *pl.* 3. (1819). (Figs. 2–S, 6).

Sphagnum miyabeanum Warnstorf, Pflanzenr. **51**: 321. *f.* 54 F. (1911).

Plant normally erect, 5–10 cm high, yellowish brown to purplish brown, in dry specimens pale green at the upper part and yellowish brown at the lower.

Wood-cylinder brown, of 4–5 layers of cells with strongly thickened walls, middle lamella deep brown, thickened layer light brown and lumens very narrow. Cortical cells of the stem commonly in a layer, occasionally intermixed with scattered parts of two layers, their walls thin, on the surface short or elongated quadrilateral, with one to two pores or thinnings of walls near the upper end of cell.

Stem-leaves triangular to triangular lingulate, often tinged with yellowish brown at the base, (0.56–) 0.70–0.90 mm long and 0.60–0.70 mm wide at the base, cucullate or narrowly truncate at the apex with minute or somewhat fimbriate teeth, the border entire, of 3–4 rows of narrow cells with pitted walls, not or slightly broadened near the base; auricles comparatively large. Hyaline cells rhomboidal in the apical part and narrowing toward the basal, often with many simple divisions, without fibrils or with fibrils only in the upper marginal part; on the inner surface with numerous large ringless pores along the commissures in the upper part, decreasing rapidly in number toward the base, cells of basal part more or less swollen and with a small pore at the upper end; on the outer surface usually without pore, but in the fibrous part with more or less numerous ringless or weakly ringed pores along the commissures.

Branches in fascicles of 4–5, 2–3 of which spreading, 7–8 (–12) mm long, usually curving downward, acute or obtuse at the apex, their cortical cells in a layer, retort-cells with inconspicuous necks. Branch-leaves ovate to ovate-oblong, 1.00–1.30 mm long and 0.60–0.70 mm wide, strongly concave, the margin strongly involute and somewhat tubular in the upper part, mucronate or very narrowly truncate at the apex with 5–10 minute teeth, the border entire, of 2–3 rows of narrow cells. Hyaline cells fibrillose, narrow vermicular, 6–8 times as long as wide in the central portion of leaf, shorter toward the apex up to 4–5 times as long, longer toward the lower and 10–11 times as long near the base; on the inner surface almost without or with a few pores, somewhat numerous in marginal regions, the pores usually ringless or weakly ringed at the upper end of cell and along the commissures, strongly ringed or bordered at the corners, besides, often with a few pseudopores or pseudopore-rudiments near the corners of cell in upper part of the leaf; on the outer surface usually with numerous pores along the commissures like a string of beads, decreasing in number toward the base, the pores round to elliptic, ringless or weakly ringed near the ends of cell, strongly ringed or bordered along the commissures, rings also becoming weak toward the base, besides, often with a few small ringless free pores in apical region.

Chlorophyll-cells in section narrow rectangular to barrel-shaped, the lumen fusiform to oblong, equally exposed on both surfaces with strongly thickened walls.

Dioecious. Antheridia in catkin on spreading branches. Perigonial leaves slightly brown, ovate oblong, strongly concave, 0.93–1.12 mm long and 0.56–0.67 mm wide, generally shorter than the normal branch-leaves

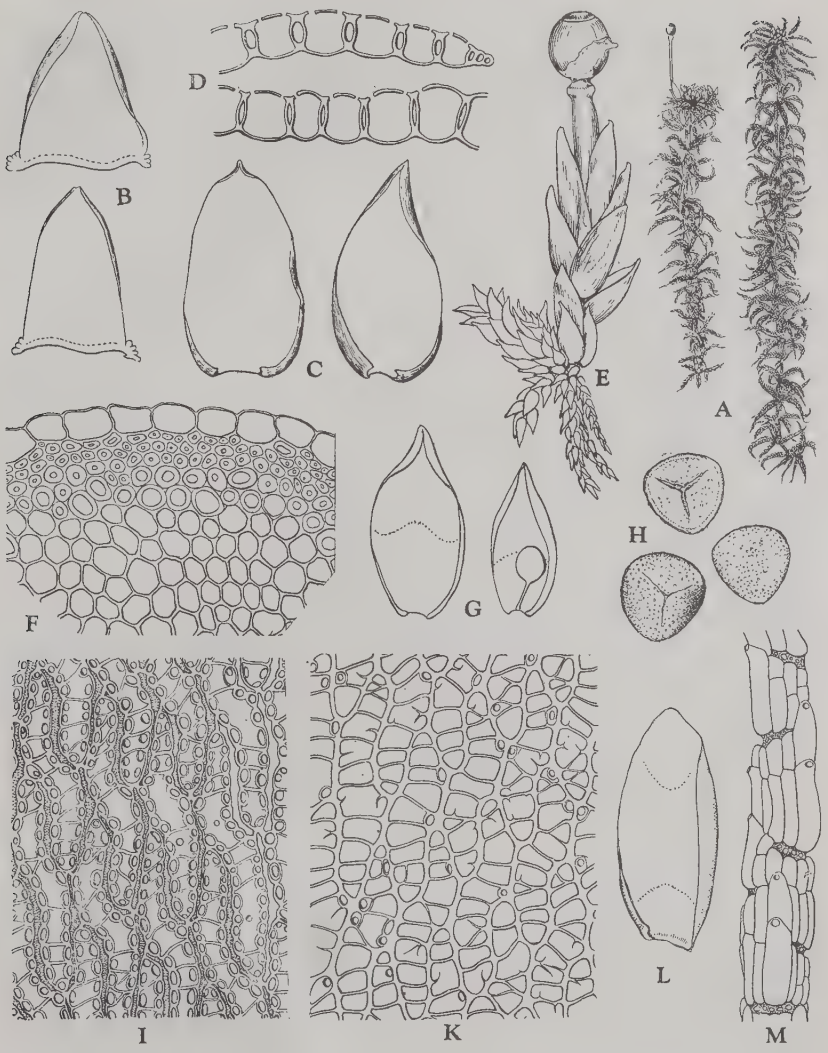


Fig. 6. *Sphagnum subsecundum* Nees.
A. Fruiting and sterile plants ×1, B. Stem-leaves ×29, C. Branch-leaves ×29, D. Cross-sections of branch-leaf ×380, E. Fruiting branch ×8, F. Cross-section of stem ×50, G. Perigonial leaves ×30, H. Spores ×447, I. Outer surface of the central portion of branch-leaf ×380, K. Inner surface of ditto ×380, L. Perichaetial leaf ×11, M. Part of denuded branch ×65. (G ... H. S. 19095, ... E.H.L. ... H.S. 21201, the others ... H. S. 22971).

and differing from the latter by the structure in basal 1/3–1/2 part, where hyaline cells commonly on both surfaces are only very finely fibrillose but often of few cells without fibrils and without pores.

Fruiting branches short or elongated, erect. Perichaetial leaves up to 10–13 in number, round ovate, often ovate lingulate or somewhat spatulate; innermost one largest, 2.3–2.7 mm long and 1.4–1.6 mm wide, obtuse or narrowly truncate at the apex, composed of two kinds of cells excepting the basal portion, the border narrow, of 3–5 rows of narrow cells with pitted walls, commonly more or less indistinct at the lower half. Hyaline cells rhomboidal near the apex, narrowing gradually toward the base, with simple or compound divisions here and there, commonly without fibrils, but in apical part of the leaf often with fine fibrils, where there are small ringless pores at the end and often along the commissures on both surfaces.

Capsule dark brown, sphaerical, 1.4–1.5 mm in diameter, with a small operculum; spores yellow, 25–28 μ in diameter, finely granular-roughened.

Nom. Jap.: *Yugami-mizugoke*.

Distribution: Iceland, Scandinavian Peninsula, Middle Europe, (in the Alps up to 2,200 m altitute), Atlantic Europe, Mediterranean Regions, (in Mts. Pyrenei 1,100–1,600 m alt. in Mts. Apenin 1,450–1,600 m alt.), Balkhan Peninsula (Yugoslavia), Russia (Arctic, European regions, Caucasia, Western Siberia, Far East), Korea (Quelpart), Japan (Hokkaido & Honshu) and North America.

Specim. exam.: **Hokkaido**, Prov. Kitami, *Rishiritonanbu*, Rishiri Isl. Oniwaki-mura, Numaura, 20m (M. Tatewaki, Sept. 26, 1952–H.S. + 15818 *S. cuspidatum*, H. Ochi-nos. 3301, 3304, July 27, 1953–H.S. 17093, 17094). Prov. Teshio, *Wakkasakinai*, Teshio-gun, Toyotomi-mura, Toyosato～Wakkasakinai, 10m, Prov. Kitami, *Hamatonbetsu*, Esashi-gun, Shimotonbetsu～Hamatonbetsu (H. Ochi. Aug. 11, 1951–H.S. 11682), *Koshimizu*, Shari-gun, Koshimizu-mura, Furutoi, 2m; Prov. Nemuro, *Attoko*, Nemuro-gun, Wada-mura, Attoko, 30m; Prov. Kushiro, *Kiritappu*, Akkeshi-gun, Hamanaka-mura, Kiritappu moor, 3.0–3.5m, *Oboro*, Kawakami-gun, Shibecha-machi, Tōro, 10m (H.S. July 22, 1956–H.S. 21180–21182 c. fr., 21186, 21187, 21188 c. anth., 21189–21191, 21201 c. fr., 21204, 21205, 21210, 21212), *Otanoshige*, ibid. Hoso-oka, 5m (H.S. July 22, 1956–H.S. 21216), Akan-gun, Tsurui-mura, Onnenai, 5m (H.S. July 21, 1956–H.S. 21164), *Shiranuka*, Shiranuka-gun, Shiranuka-mura, Shoro (K. Ito, July 25, 1950–H.S. 10390–b + *S. subobesum*), ibid. 5m (H.S. July 19, 1956–H.S. 21124 + *S. obtusum*); Prov. Tokachi, *Urahoro*, Nakagawa-gun, Toyokoro-mura, Toyokoro, 13m (H.S. July 23, 1956–H.S. 21220–b, 21225–a, 21233, 21234, 21242, 21245–a + *S. kushiroense*, 21246, 21248). Prov. Ishikari, *Asahidake*, Kamikawa-gun, Higashikawa-mura, Mts. Daisetsu, Hyotan-numa, 930 m; Biei-machi, Mt. Tomuraushi, Kita-numa; ibid. Numanohara-moor, 1440–1460m (H. S. Aug. 27, 1952–H. S. 14033), ibid. Tenjinkyo～Yukomanbetsu, 980m (H.S. Aug. 13, 1954–H.S. 18468 + *S. papillosum*); *Kokuryo*, Uryu-gun, Uryu-numa moor, 850m (M. Saito-nos. 23786, 23848, 23858, 23868, Aug. 9, 1955–H.S. 20454, 20457–b, 21433, 21450), *Sunagawa*, Bibai-shi, Bibai plain, Sakura-numa (Bake-numa), 20m; *Iwamizawa*, ibid. about the Bibai Peat Soil Experimental Farm., 15–18m (H.S. Aug. 24, 1954–H.S. 19074, 19075, 19083), ibid. noth of Nimai-bashi, 13–14m (H.S. Aug. 25, 1954–H.S. 19181); *Tōbetsu*, Sorachi-gun, Kita-mura～Iwamizawa, Akagawa, 11m; Ishikari-gun, Shinshinotsu-mura, Shinotsuno plain, 11–12m, *Ebetsu*, Iwamizawa-shi, Horomui plain, 9m; Prov. Iburi, *Oshamanbe*, Yamakoshi-gun, Oshamanbe-machi, Shizukari-plain, 7.5m (H.S. Sept. 11, 1952–H.S. 14595, 14596, 14604).

N. E. Honshu, Prov. Mutsu, *Hakkodasan*, Higashitsugaru-gun, Mt. Hakkoda, Senninta, 1250m (H.S. Aug. 10, 1940–H.S. 22971, Y. Horikawa, Aug. 17, 1948–H.S. 2666, H.S. Aug. 22, 1949–H.S. 7424 + *S. papillosum*, 7427, 7440, H. Tamura, July 23, 1949–H.S. 12699, 12701, H. Ando-no. 14243, July 22, 1953–H.S. 17623).

M. Honshu, Prov. Iwashiro, *Fujiwara*, Minamiaizu-gun, Hinoemata-mura, Ozegahara, Shimotashiro 1410m; Prov. Shinano, *Shiroumadake*, Kitaazumi-gun, Minamiotari-mura, Mt. Shōrenge-yama, Ō-ike, 2380m (N. Takaki-no. 7034 p.p., Aug. 1, 1949–H.S. 7668-b).

At present this species is known from about 29 localities in Japan; viz. 23 in Hokkaido, four in N.E. Honshu and two in M. Honshu. In Hokkaido, the species occurs widely not only in the lowlands but also in mountainous regions. It ascends up to 1,460 m in altitude in Numanohara moor, central part of Hokkaido, but most localities in this island are situated in the plain lower than 30m in elevation and the lowest station is a coastal plain at Furutoi, on the Sea of Okhotsk, where it descends to about sea-level. On the other hand, all the localities in Honshu are situated in the high mountains; viz. from north to south, Mt. Hakkoda (ca. 980–1,300 m alt.) Ozegahara moor (ca. 1,410 m) and Mt. Shōrengeyama (ca. 2,380 m). Generally speaking, the more southern locality has the higher elevation, and the abundancy of this species decreases abruptly toward the southern localities. On Mt. Hakkoda, located near the northern end of Honshu, it is observed to grow laxuriantly in four peat-bogs developing between 980 m and 1,300 m in elevation (Suzuki, 1941, 1948). A few examples of this species were found in a packet of *S. compactum* from Ozegahara moor (Horikawa and Suzuki, 1954). Only a few plants of this species were found in a packet of *S. robustum* collected by one of my friends Dr. N. Takaki from Mt. Shōrengeyama, located in the central part of Honshu, and this is southernmost (36° 47′ N.L.) and the highest locality (2,380 m) of this species in Japan. It is clear from both its peculier range in the world and its vertical and horizontal distributions in Japan that this species is one of the arcto-alpine elements.

4. Sphagnum contortum Schultz, Fl. Starg. Suppl. 64. (1819). (Fig. 7).

Plant rather small, slender or robust, erect or decumbent, 4–10 cm high, yellowish green, yellowish brown to dark purplish brown.

Wood-cylinder brownish yellow to reddish brown, without thickened layer in cell-wall; cortical cells commonly in 2 layers, often partially in 1 or 3 layers, cells of inner layer somewhat smaller, on the surface short or elongated quadrilateral, usually with a large pore or thinning of wall near the upper end of cell.

Stem-leaves triangular lingulate, (0.56–) 0·63–0.93 (–1.05) mm long and (0.41–) 0.45–0.65 (–0.71) mm wide at the base, usually cucullate or roundly truncate with minute teeth at the apex, margin involute in the upper part, the border entire, of 2–7 rows of narrow cells, narrowing toward both base and apex, usually somewhat hyaline near the apex; auricles small. Hyaline cells rhomboidal near the apex, narrower and longer toward the base,

with simple or compound divisions, especially in apical region, fibrillose only at apical part, where there are numerous large ringless pores along the commissures on both surfaces and usually more numerous on the inner surface, cells just above the base somewhat swollen and with a small pore near the upper end.

Branches in fascicles of 4–5, rarely 6, 2 or rarely 3 of which spreading, up to 9–11 mm long, more or less thick and arcuate, smooth or somewhat serrate on the surface, obtuse or attenuate at the apex; their cortical cells in a layer, retort-cells with inconspicuous neck and commonly 2 in a row. Branch-leaves ovate to ovate lanceolate, usually somewhat asymmetrical,

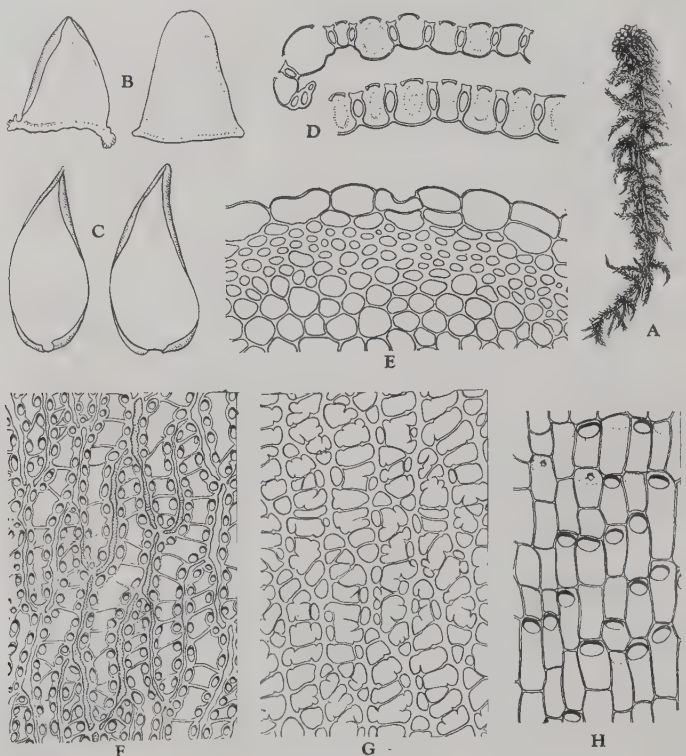


Fig. 7. *Sphagnum contortum* Schultz
A. Sterile plant ×3/4, B. Stem-leaves ×23, C. Branch-leaves ×23, D. Cross-sections of ditto ×335, E. Part of cross-section of stem ×235. F. Outer surface of the central portion of branch-leaf ×335, G. Inner surface of ditto ×335, H. Outer surface of stem ×235. (H. S. 5882)

strongly concave, (1.00–) 1.30–1.60 (–2.00) mm long and (0.56–) 0.60–1.00 (–1.12) mm wide, cucullate or narrowly truncate at the apex with 5–7 incurved teeth, the margin stronly involute, often somewhat tubular in the upper part, the border narrow, entire, of 2–3 rows of narrow cells with pitted walls. Hyaline cells vermicular, fibrillose on both surfaces, 6–7 times as long as wide in the middle portion, shorter toward the apex up to 5–7 times as long and somewhat larger below and 6–9 times as long near the base; on the inner surface almost without pores, and usually with more or less numerous pseudopores and pseudopore-rudiments along the commissures, but in marginal part of the leaf often with a few large ringed pores; on the outer surface with numerous large ringed pores along the commissures like a string of beads, besides, occasionally here and there with some free ringless pores. The leaves of pendent branches similar to those of spreading.

Chlorophyll-cells in section barrel-shaped with oblong to fusiform lumens, exposed more widely on the outer surface, with thick walls on both surfaces.

Sexual organs or sporophytes are not found from Japan.

Nom. Jap.: *Nejire-mizugoke.*

Distribution: Scandinavian Peninsula (up to 67° 40′ N.L.), Central Europe (in the Alps up to 2,000m alt.), Atlantic Europe, Balkhan Peninsula, Russia (Arctic, European regions, Caucasia, Western & Eastern Siberia and Far East), Japan (Honshu) and North America.

Specim. exam.: **M. Honshu**, Prov. Shinano, *Suwa*, Suwa-shi, Mt. Kirigamine, Yashimagahara moor, 1650m (T. Seki-nos. 6685, 6687, 6690, Aug. 15, 1956–H.S. 21592, 21628, 21629), ibid. Yashimaga-ike, 1650m (H.S. July 13, 1949–H.S. 5804), ibid. Kamaga-ike, 1650m (H.S. July 13, 1949–H.S. 5867, 5869, 5871+*S. guwassanens* ssp. *takedae*, 5874-a), ibid. western part of the moor, 1650m (H.S. July 26, 1954–H.S. 18114, 18115+*S. papillosum*, 18116+*S. papillosum*, 18118, 18119), ibid. Ko-ike, 1650m (H.S. July 26, 1954–H.S. 18149+*S. guwassanense* ssp. *takedae*, 18151+*S. jensenii*, *papillosum* and *amblyphyllum*), ibid. southern part of the moor, 1640m (H.S. July 26, 1954–H.S. 18178, 18179), ibid. a bog east of Yashimagahara moor, 1720m (H.S. July 13, 1949–H.S. 5880, 5882–5884, 5886, 5888), ibid. Odoriba～Kurumayama, 1670m (H.S. July 14, 1949–H.S. 5905), ibid. Kurumayama moor, 1850 m (H.S. July 14, 1949–H.S. 5933-a).

As was mentioned above that this species is found only on Mt. Kirigamine located in the central region of Honshu, it grows most abundantly at Yashimagahara moor and at three other peat-bogs, that lie between 1,640 m and 1,850 m in elevation on this mountain.

5. Sphagnum microporum Warnst. ex Cardot, Beih. Bot. Centralbl. **17**: 3. *f.* 1. (1904). (Fig. 8).

Sphagnum oligoporum Warnst. & Cardot ex Cardot, Bull. Herb. Boiss. Ser. 2. **7**: 711. (1907).

Sphagnum okamurae Warnstorf, Hedwigia **47**: 97. (1907).

Sphagnum inundatum (Russ. p.p.) Warnstorf, quoad plant. jap. p.p.

Plant rather soft and robust, (3–) 5–10 (–12) cm high, normally deep green to light yellowish green and occasionally dark brown in the upper

part, discolored and pale green below and dark yellowish brown at the base.

Wood-cylinder yellow to yellowish brown, comparatively thin, of 2–4 rows of cells with thick wall; cortical cells commonly in a layer, very rarely 2 in part, the walls thin, on the surface short quadrilateral with 4–6 angles, with one or two irregular openings or thinnings of wall.

Stem-leaves short triangular lingulate to lingulate, strongly concave, (0.86–) 0.90–1.00 (–1.16) mm long and (0.45–) 0.60–0.70 mm wide at the base, often cucullate at the apex where it is somewhat fimbriate or denticulate or roughly dentate, the margin involute in the upper half, the border, narrow, entire, of 2–3 (–6) rows of narrow cells, not broadened toward the base, usually more or less hyaline near the apex; auricles rather small. Hyaline cells rhomboidal near the apex, longer and broader toward the base, fibrillose on both surfaces in upper 1/3–1/2 or down to much lower part of the leaf, commonly with numerous simple or compound divisions, particularly in the fibrous part; on the inner surface the pores numerous in fibrous part, generally decreasing in number toward the base, various in size, scattered near the commissures, ringless to strongly ringed, often bordered near the corners of cells, in the lower fiberless part usually ringless at both ends and corners of cells, in basal part small and only one at upper end, in apical part often small ringless and free in 1–2 rows; on the outer surface generally similar with those on the inner surface, but less numerous in number, almost lacking in the fiberless part, but occasionally dense in very apical part like a sieve.

Branches in fascicles of 4–5 (–6), often with secondary branches, 2–3 of which spreading, generally arcuate, attenuate or somewhat obtuse at the apex, 8–10 (–13) mm long, their cortical cells in a layer, the retort-cell with inconspicuous neck, usually 2–3 in a row, rarely single, often intermixed with a small cell in its row.

Branch-leaves ovate to ovate lanceolate, somewhat asymmetrical, very strongly concave, ordinarily more or less falcate, 1.50–1.68 mm long and 0.75–0.90 mm wide at the base, narrowly truncate and often somewhat cucullate at the apex with 3–5 (–7) incurved teeth, the margin strongly involute in the upper part, the border entire, rather broad, of 3–5 rows of narrow cells, not broadening toward the base. Hyaline cells with numerous fibrils on both surfaces, vermiform, 5–6 times as long as wide in the middle portion, shorter and narrower toward the apex up to 7–10 times as long, slightly larger below and 6–8 times as long near the base; on the inner suface ordinarily almost without pores or with a few pores, rarely with somewhat numerous pores, but generally less numerous than on the outer surface, the pores small, ringless to ringed or bordered, mainly situated at the ends and corners of cell, in multiporous leaves the pores situated also along the commissures and usually increase in number toward the lower marginal portion; on the outer surface commonly numerous pores arranged in discontinuous rows along the commissures, decreasing in

Fig. 8. *Sphagnum microporum* Warnst. ex Cardot
A. Fruiting and sterile plants ×3/4, B. Branch-leaves ×23, C. Stem-leaves ×23, D. Cross-sections of branch leaf ×335, E. Perichaetial leaf ×8, F. Spores ×335, G. Fruiting branch ×5, H. Outer surface of the apical part of stem-leaf ×235, I. Inner surface of ditto ×235, K. Cross-section of stem ×110, L. Outer surface of stem ×110, M. Outer surface of the central portion of branch-leaf ×335, N. Inner surface of ditto ×335, O. Inner surface of the apical part of perichaetial leaf ×235, P. Part of denuded branch ×58. (H. S. 20817).

number toward the base and often remote from the commissures, the pores small, generally weakly ringed, often strongly ringed or bordered at the ends or corners of cell, very rarely intermixed with few pseudopores.

Chlorophyll-cells in cross-section trapezoid to trapezoidal barrel-shaped, more broadly exposed on the outer surface with more or less thickened walls.

Dioecious. Antheridia in catkin on spreading branches, rarely on pendent ones. Perigonial leaves brown, shorter than normal branch-leaves with obtuse apex, elliptical, or ovate oblong, strongly concave, especially near the base, up to 1.12 mm long and 0.67 mm wide, slightly differing from the normal branch-leaves by lacking pores on the outer surface in basal half part where there are more or less fine fibrils or often without fibrils in a few cells. Fruiting branches usually elongated, erect. Perichaetial leaves up to 12 in number, innermost one largest, 3.36–3.41 mm long and 2.00–2.27 mm wide, acute and minutely denticulate or somewhat fimbriate at the apex, with narrow borders, of 2–7 rows of narrow cells with pitted walls, entirely composed of two kinds of cells. Hyaline cells rhomboidal, almost of equal size, often with simple or compound divisions, with fine fibrils all over or scattered on the leaf; on both surfaces the pores more or less remote from the commissures and decreasing below and lacking near the base, on the inner surface numerous small, ringless and situated at the ends and corners of cell or along the commissures, more numerous than on the outer.

Capsule dark brown, elongated sphaerical, 1.3–1.5 mm in diameter, with small operculum; spores yellow, 20–25 μ in diameter, finely granular-roughened.

Nom. Jap.: *Koana-mizugoke*, (*Koma-mizugoke*, *Sendai-mizugoke*, *Kazusa-mizugoke* p.p.)

Distribution: China, North Korea and Japan (Honshu, Shikoku and Kyushu).

Specim. exam.: **N. E. Honshu**, Prov. Mutsu, Aomori, (Faurie-no. 3, Sept. 1902, as *S. inundatum*, in KYO*); Prov. Rikuzen, Matsushima michi (E. Ishiba, May 2, 1907–Okamura no. 32, as *S. okamurae*, in NICH*), *Sendai*, Sendai-shi, (E. Uematsu, Oct. 17, 1906–Okamura no. 16, isotype of *S. okamurae*, in NICH), ibid. Miyagi-gun, Aoso (Nakayamafudō), 90m (E. Uematsu, Apr. 12, 1908–Okamura no. 58, as *S. okamurae*, in NICH), ibid. near Tōshōgu, 40m (E. Ishiba, May 24, 1907–Okamura no. 35, as *S. okamurae*, in NICH), ibid. Naga-machi (as Natori-gun, Mogasaki-mura) (E. Uematsu, May 3, 1908–Okamura no. 59, isotype of *S. okamurae* var. *robustum*, in NICH), ibid. Anyoji, Yoheinuma, 50m (A. Miyawaki, Apr. 20, 1955–H.S. 19856); Prov. Echigo, *Katsugi*, Iwafune-gun, Asahi-mura, Bunda, 350m (Y. Ikegami-nos. 43801–43803, Aug. 30, 1956–H.S. 21841–21843); *Nakajō*, Iwafune-gun, Kanaya-mura, Nakano, 8–9m (Y. Ikegami-nos. 24770, 24776, 24775, Nov. 4, 1952–H.S. 20282, 20287, 20289).

M. Honshu, Prov. Echigo, *Niigata*, Nakakanbara-gun, Ōeyama-mura, Komagome, 3m (Y. Ikegami-no. 24626, Sept. 18, 1952–H.S. 21237), *Shibata*, Kitakanbara-gun, Seiro-mura,

* KYO and NICH show that the speciemen is deposited in the herbarium of the Kyoto University and in that of the Hattori Botanical Laboratory respectively.

Hasuno, 3m (Y. Ikegami-nos. 24672–24675, Oct. 4, 1952–H.S. 20238–20241), ibid. Hondamura, Tsukioka, 40m (Y. Ikegami-no. 35055, Oct. 17, 1954–H.S. 21796), *Niitsu*, Kitakanbara-gun, Bunda-mura, Yamamotoshin, 20 m (Y. Ikegami –no. 24692, Oct. 11, 1952 H.S. 20249); Prov. Iwashiro, *Sukagawa*, Iwase-gun, Kagamiishi-mura, Kasaishi, 276m (H.S. July 29, 1956–H.S. 21326, 21327, 21335); Prov. Iwaki, *Tanagura*, Nishishirakawa-gun, Sekihira-mura, Shinbayashi, 300m (H.S. Dec. 8, 1947–H.S. 1218), ibid. Kamako-mura, Kabunchi (R. Watanabe, Apr. 12, 1949–H.S. 7679), *Shirakawa*, Nishishirakawa-gun, Kotagawa-mura, Izumida, 350m (H.S. Dec. 3, 1947–H.S. 1214–1216), ibid. Shirasaka-mura, near Station, 400m (Y. Ikegami-nos. 34556, 34557, Aug. 12, 1954–H.S. 21876, 21877), Shirakawa-shi, Nanko Park, 350m (H.S. Dec. 4, 1947–H.S. 1181, Y. Ikegami-no. 33897, Aug. 6, 1954–H.S. 20329, H.S. July 9, 1956–H.S. 20817 c. fr., 20818–20822, 20825), ibid. Nanko Pasture, 360m (H.S. Dec. 4, 1947–H.S. 1201–1207, 1191–1193, 1197, 1198); Prov. Kōzuke, *Numata*, Seta-gun, Mt. Akagi, Daido, Manbuchi, 1360m (Y. Haraguchi-nos. 16, 17, 20, 22, July 27, 1953–H.S. 17177, 17178, 17181, 17183 c. anth.); Prov. Kazusa, *Mohara*, Mohara-shi, (M. Gōno. Oct. 14, 1906– in Okamura's collection as *S. inundatum*, in NICH, M. Ōki-no. 35, May 31, 1935–H.S. 15793); Prov. Shinano, *Karuizawa*, Mt. Asama (Faurie no. 77, July 21, 1897, as. *S. inundatum*, in KYO), *Tateshinayama*, Minami-saku-gun, Mt. Yatsugatake, Umiziri~Higashizawa-kōsen, 1150m (S. Nakanishi, July 12, 1952–H.S. 13148), *Suwa*, Suwa-shi, Mt. Kirigamine, Yashimagahara moor, 1640m (N. Takaki-no. 6531, June 27, 1949–H.S. 8399, T. Seki-nos. 6640, 6641, Aug. 15, 1956–H.S. 21161, 21162), ibid. southern part of the moor, 1640m (H.S. July 26, 1954–H.S. 18156, 18158, 18160-b, 18177), ibid. a bog east of Yashimagahara moor, 1720m (H.S. July 13, 1949–H.S. 5881, 5885), ibid. Kurumayama moor, 1850m (H.S. July 14, 1949–H.S. 5922, 5927, 5928, 5931, 5935, 5941, Y. Kubota-nos. 270, 319, June 30, 1950–H.S. 20380, 20391), ibid. 1760–1780m (T. Seki-nos. 6666, 6667, 6681, Aug. 15, 1956-H.S. 21608, 21609, 21612), Suwa-gun, Kitayama-mura, Shirakabako, 1430m (H.S. July 27, 1954–H.S. 18207, 18216, 18217, 18220, 18221, 18225), *Ontakesan*, Mt. Ontake (Faurie-no. 217, Sept. 1905, as *S. inundatum*, in KYO), *Tsumago*, Nishichikuma-gun, Yomikakimura, Sodeyama, 1000m (Y. Sasaki-no. 645, 647, Aug. 6, 1949–H.S. 7691, 7692, H.S. July 27, 1950-H.S. 10371–10376, 10378, 10388); Prov. Mikawa, *Miabura*, Minamishidara-gun, Shinshiro-machi, Sugiyama, 50m (M. Fukuhara-N. Takaki-no. 6215, May 18, 1949–H.S. 5278), *Toyohashi*, Hazu-gun, Futakawa-machi, Fumonji, 100m (Y. Tatsukawa-N. Takakino. 5588, Oct. 3, 1948–H.S. 4542); Prov. Mino. *Gifu*, Gifu-shi, (as Inaba-gun, Hino-mura), Funabuseyama (Hama, Dec. 8, 1908–Okamura no. 99, isotype of *S. okamurae* var. *angustifolium*, in NICH).

S. W. Honshu, Prov. Iga, *Yokkaichi*, Mie-gun. Kanzaki-mura, Ōike, 45m (H. Ando. Aug. 17, 1951–H.S. 12429, 12430); Prov. Yamashiro, *Kyōtotōhokubu*, Kyōto-shi, Kamigyōku, Kamigamo, Midoroga-ike, 75m (H.S. May 1, 1955–H.S. 19883–19887 c. anth., 19888 c. anth., 19889, 19894, 19896–19900, 19903, 19904, 19910); Prov. Settsu, *Sonobe*, Toyono-gun, Nishinose-mura, Tennō, 570m (T. Nakajima-no. 7110, Apr. 5, 1956–H.S. 20465); Prov. Izumi, *Kishiwada*, Senhoku-gun, Minamiikeda-mura, Nōke (T. Nakajima-no. 980, Apr. 22, 1951–H.S. 14983); Prov. Mimasaka, *Yumoto*, Moniwa-gun, Kawakami-mura, Kamihirusen, Mikigahara, 450–500m (A. Miyawaki, Aug. 29, 1950–H.S. 10750, H. Takemoto, Sept. 5, 1956–H.S. 21430), ibid. Yatsuka-mura, Kamihirusen, 450–500m (H. Takemoto, Sept. 5, 1956–H.S. 21434–21440), ibid. Shimohirusen, 450m (C. Igi-nos. 2457, 2458, Sept. 5, 1954–H.S. 21399, 21400), ibid. south of Inubasami Pass, 500m (H. Takemoto, Sept. 5, 1956–H.S. 21433); Prov. Bitchu, *Kamiiwami*, Atetsu-gun, Niizato-mura, Nabara, 550m (C. Igi-no. 94, Sept. 8, 1946–H.S. 21397); Prov. Bingo, *Tari*, Hiba-gun, Saijō-machi, Mt. Tateebōshi, Ikenodan, 1220m (K. Negayama-nos. 32, 34, 36, Aug. 22, 1955–H.S. 20101, 20103, 20104, H.S. May 30, 1957-H.S. 22444, 22445, 22450, 22452, 22456), *Kamifuno*, Hiba-gun, Yamanouchihigashimura, Yoshii, 250m (H.S. Apr. 17, 1949–H.S. 5231–5242), Futami-gun, Kimita-mura,

Higashiirigimi, 380m (H.S. Nov. 14, 1948–H.S. 4440–4444), *Miyoshi*, Hiba-gun, Yamanouchi-higashi-mura, Nanatsukahara, 300m (H.S. Apr. 12, 1948–H.S. 1360, 1361), ibid. 310m (H.S. Apr. 12, 1948–H.S. 1364–1367), ibid. Yamanouchinishi-mura, Ōhara, 270m (H.S. 12, 1948–H.S. 1355–1359), Futami-gun, Sakegawa-mura, south of Minosuke-ike, 200m (H.S. Feb. 13, 1948–H.S. 1320–1325), ibid. Higashisakeya, 250m (H.S. Dec. 26, 1947–H.S. 1304–1306). Tōkaichi-machi, Yatsugi, (H.S. May 16, 1947–H.S. 53, 54 c. anth. 55, 72, 73, H. Ando, May 13, 1951–H.S. 12818), ibid. southeast of Minosuke-ike, 180m (H.S. May 6, 1947–H.S. 6), south of ditto, (H.S. Oct. 4, 1947–H.S. 1115), ibid. Kasumiga-ike, 180m (H.S. May 9, 1947–H.S. 15, 16, Sept. 7, 1947–H.S. 939, 950-a, -b, -c, 960, Apr. 30, 1950–H.S. 8277), *Nomi*, Sera-gun, Kanda-mura, Hongō, 480m (H.S. Dec. 4, 1954–H.S. 19802), ibid. Shimotokura, Kameyamahachi-man, 350m (H.S. Dec. 5, 1954–H.S. 19787–19789, 19790 c. anth. 19791. 19792 c. anth. 19793, 19798, 19799); Prov. Aki, *Kitsuga*, Yamagatagun, Ogawara-mura, Tōrabara, 600m (Y. Watanabe, Aug. 10, 1951–H.S. 12434–12442, 12444), ibid. Yawata-mura, Ozakidani, 780–790m (H.S. Nov. 23, 1953–H.S. 17588, 17589, 17592–17594, Nov. 7, 1954–H.S. 19616, Aug. 16, 1956–H.S. 21373, 21374), ibid. north of the Primary School, 780m (H.S. Nov. 5, 1954–H.S. 19594, 19597–19600), ibid. Senchōbara, 810m (H.S. Aug. 14, 1955–H.S. 20085, 20086).

Shikoku, Prov. Iyo, *Matsuyamananbu*, Onsen-gun, Kume-mura, Higashino (Shibagatōge), 160–170m (S. Yamamoto, Jan. 15, 1953–H.S. 15021, 15022, T. Seki-no. 2492, July 15, 1956–H.S. 21488, July 20, 1956–H.S. 21418–21420); Prov. Tosa, *Ino*, Kōchi-shi, Asakura-machi, Ebikawa, 80m (T. Yamanaka, July 8, 1950–H.S. 10737, 10738, H.S. May 11, 1953–H.S. 15044).

Kyushu, Prov. Bungo, *Beppu*, Ōita-gun, Yunohira-mura, Odano-ike, 750m (M. Arakane, July 10, 1956–H.S. 20572, 20574–20584, Oct. 10, 1956–H.S. 21456–21459, Aug. 18, 1956–H.S. 21460, 21461, July 8, 1956–H.S. 21462–21469), ibid. Yamashitano-ike, 750m (M. Arakane, Aug. 18, 1956–H.S. 21470).

At present this species is known from approximately 61 localities in Japan; viz. 8 in N.E. Honshu, 24 in M. Honshu, 25 in S.W. Honshu, two in Shikoku and two in Kyushu. It is interesting to note that most of these localities are situated in middle and southwestern Honshu. According to the writer's present knowledge, the northernmost limit of the distribution lies near Aomori (45° 50′ N. L.)and the southernmost is Odano-ike (33° 12′ N.L.) located in the northern part of Kyushu. The highest elevation is the Kurumayama moor on Mt. Kirigamine where it ascends to 1,850 m and the lowest is Komagome and Hasuno, both located in the Japan Sea coastal plain of the middle Honshu, where it descends to about sea-level. From the range of its vertical and horizontal distributions, it may be conjectured that this species has its center of distribution in southwestern Honshu. It is, moreover, very interesting from the plantgeograpical point of view that this species was originally described based on the specimen collected from Ouen-San (Wonsan) in North Korea and *S. oligoporum*, treated by the writer as conspecific with this species, has recently been reported from the eastern part (Chiangsu) of China (Chen & Lee, 1956).

6. Sphagnum kushiroense H. Suzuki sp. nov.

Planta mediocris, erecta, plus minusve robusta, rigida, 5–10 cm alta, pallide viridis, flavo-virens, flava vel flavo-brunnea, deorsum pallescens. Cylindrus ligosus pallens, fulvus, saepe rufus e 3–5 atratis cellularum sclerenchymatarum formatus. Hyalodermis caulis strato uno, rarissime

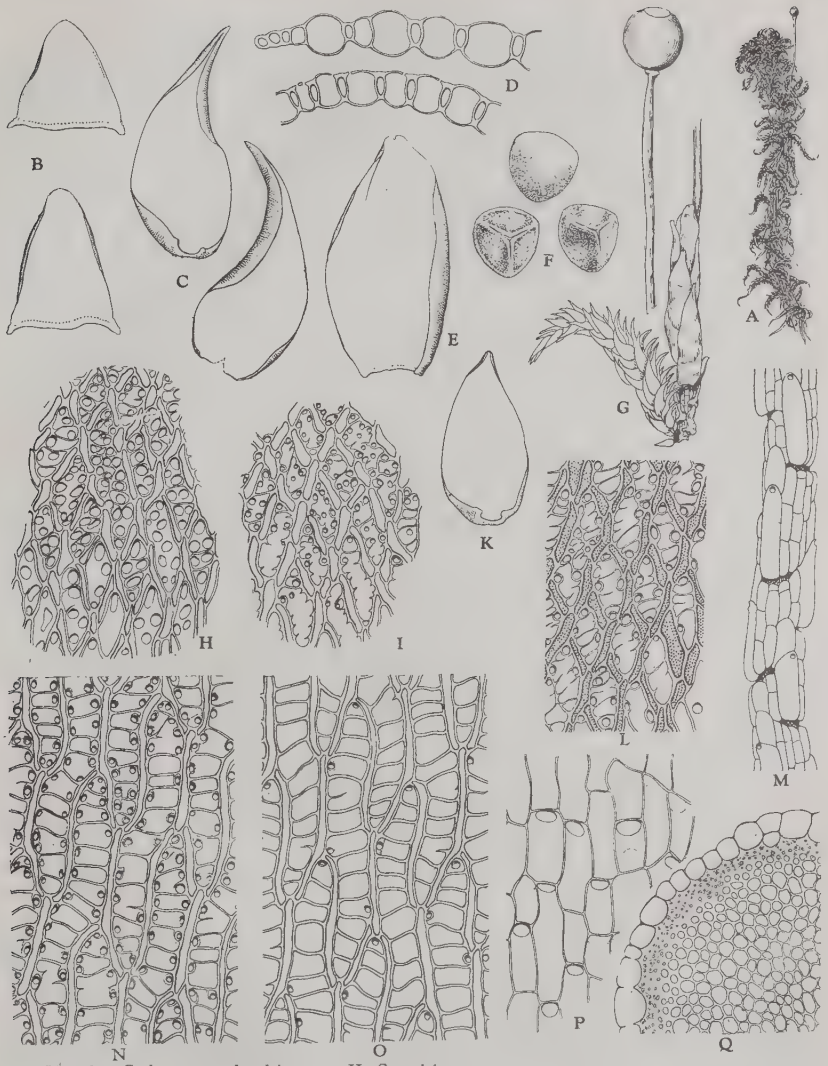


Fig. 9. *Sphagnum kushiroense* H. Suzuki
A. Fruiting plant ×3/4, B. Stem-leaves ×23, C. Branch-leaves ×23, D. Cross-section of ditto ×335, E. Perichaetial leaf ×8, F. Spores ×335, G. Fruiting branch ×5, H. Inner surface of the apical part of stem-leaf ×235, I. Outer surface of ditto ×235, K. Perigonial leaf ×23, L. Inner surface of the apical part of perichaetial leaf ×235, M. Part of denuded branch ×58, N. Outer surface of the central part of branch-leaf ×335, O. Inner surface of ditto ×335, P. Outer surface of stem ×110, Q. Cross-section of stem ×110. (K ... H. S. 14521, the other ... H. S. 21127).

sparsim duobus; cellulis superficie breviter vel longe quadrilateralibus, parietibus primo aporosis, deinde saepe extremo tantum supremo raro utroque poro uno magno vel partibus tenuibus poriformibusque evolutis, demum parietibus plerumque dissolventibus.

Folia caulina minuta, plerumque anisophylla, breviter deltoideo-lingulata, (0.63–) 0.80–1.00 (–2.00) mm longa, ad basin (0.52–) 0.60–0.70 (–0.80) mm lata, apice vulgo cucullata, subfimbriata, anguste limbata, limbis e 2–5 seriebus cellularum angustissimarum formatis, superne aliquantum hyalinis, basin versus vix vel leviter dilatatis. Cellulae hyalinae in superioribus partibus rhomboideae, inferioribus elongatae, saepe uni- vel biseptatae, in parte apicali foliorum fere efibrillosae vel paulum fibrillosae; interiore folii superficie poris magnis irregularibus non-annulatis, versus dimidiam partem inferiorem in numero deminuentibus praeditae; dorso foliorum prope apicem solum minute porosae, poris in cellularum angulis vulgo annulatis vel limbatis.

Rami in fasciculo 5–6, saepe 4 vel 7, quorum 2–3 patentes, ad 1.6 mm longi, attenuati, plerumque arcuati ad curvati, hyalodermide strato uno, cellulis retortiformibus soritariis cervicibus inconspicuis. Folia ramulina ovato-lanceolata, concava, superne secundo-falcata, magnitudine varia, 1.50–2.50 mm longa, (0.60–) 0.70–0.90 (–1.12) mm lata, apice in expansione anguste truncata, saepe plus minusve cucullata, incurve paucidenticulata, marginibus superne incurvatis integrisque, anguste limbata, limbis e 2–3 seriebus cellularum angustissimarum compositis. Cellulae hyalinae vermiculatae, multifibrosae, ad medium folii 10–13–plo longiores quam latiores, sursum angustiores tum 14–15–plo longiores quam latiores, deorsum latiores et ad basin folii 5–7–plo longiores quam latiores; interiore folii superficie fere aporosae vel pauciporosae, poris non-annulatis in parte ultima cellularum, plus minusve annulatis in cellularum angulis, in marginalibus partibus inferioribus foliorum annulatis et numerosis ad commissuras: dorso poris vulgo multis, non-annulatis vel subannulatis ad limbatis, in seriebus interruptis ad commissuras dispositis, versus basin folii in numero deminuentibus.

Cellulae chlorophylliferae sectione transversali plerumque trapezoideae ad orciformes, cum pariete exteriore longiore incrassatoque dorso foliorum sitae, utrinque liberae.

Dioicum. Antheridia in ramulis patentibus amentaceis sita. Folia perigonalia ovata, fulva, vix subsecunda, valde concava, 1.12–1.20 mm longa, 0.52–0.60 mm lata, apice rotundato-truncata, potius late limbata, limbis e 3–5 seriebus cellularum angustissimarum formatis. Cellulae hyalinae in parte inferiore perigoniorum foliorum vulgo tenuiter fibrillosae vel saepe in cellulis paucis efibrillosae et in tantum extremo supremo vel utroque poris exannulatis praeditae, quae notae ab iis in foliis ramulinis normalibus discrepantes.

Ramus fructifer vulgo elongatus, erectus. Folia perichaetialia ad 12, intimum maximum, 3.7–4.0 mm longum, 2.22–2.50 mm latum, apice emarginatum, anguste limbatum, limbo e 2–5 seriebus cellularum angustissimarum

formato, parte basilari excepta cellulis biformibus compositum. Cellulae hyalinae rhomboideae, per totum folium fere aequiformes et paene efibrillosae, sed parte superiore vel in cellulis paucis sparsis satis tenuiter fibrillosae, in parte folii superiore uni- vel multiseptatae; superficie interiore in extremis cellulae poris annulatis exannulatisve et in parte apicali folii praeterea poris saepe ad commissuras praeditae; dorso poris solum in cellulis paucis prope apicem folii vel raro etiam in regionibus superioribus marginalibusque. Capsula atro-fusca, globosa, 1.87–1.95 mm in diametro, operculo parvo; sporae fulvae, 30–32 μ in diametro, minute granulatae.

Nom. Jap.: *Kushiro-mizugoke*.

Distribution: Endemic to Japan (Hokkaido).

Specim. exam.: **Hokkaido,** Prov. Kushiro, *Oboro*, Akkeshi-gun, Akkeshi-machi, Kamioboro, 45 m (H. S. Sept. 6, 1952–H. S. 14521 c. anth.–paratype in HIRO), Kawakami-gun, Shibecha-machi, Tōro, 10 m (H. S. July 22, 1956–H. S. 21184 c. fr, 21196–a), *Otanoshige*, Akan-gun, Tsurui-mura, Onnenai, 5 m (H. S. July 21, 1956–H. S. 21168). *Shiranuka*, Shiranuka-gun, Shiranuka-machi, Shoro (K. Itō, July 25, 1950–H. S. 10390–a), ibid. 5 m (H.S. July 19, 1956–H.S. 21124, 21127, c. fr.–holotype in HIRO, 21135), *Onbetsu*, Shiranuka-gun, Onbetsu-mura, west of Mashikuru-numa, 10 m (H. S. July 19, 1956–H. S. 21112, 21114); Prov. Tokachi, *Urahoro*, Nakagawa-gun, Toyokoro-mura, Toyokoro, 13 m (H. S. July 23, 1956–H. S. 21225–b, 21229); Prov. Oshima, *Ōnumakōen*, Kameta-gun, Nanae-mura, Ōnuma Park, south of Ōnuma, 145 m (H. S. Aug. 11, 1954–H. S. 18347, 18348, 18350).

This species is closely allied to *S. microporum* and it is difficult to distinguish from each other in sterile plants, as they chiefly differ in sexual organs or sporophytes. The present species has larger sphaerical capsule and larger spores than *S. microporum*. Anisophyllous stem-leaves in this species serve also to distinguish it from *S. microporum* in which they are rather rare. At present, the species is restricted to the south coastal regions of Hokkaiko.

7. Sphagnum subobesum Warnstorf, Hedwigia **39**: 104. (1900). (Figs. 1, 2–O & 10).

Sphagnum uzenense Warnst. in litt. (1909) & Pflanzenr. **51**: 394. f. 65 B. (1911).

? *Sphagnum microporum* Warnst. var. *junsaiense* Warnst. Pflanzenr. **51**: 314. (1911).

Sphagnum inundatum (Russ. p.p.) Warnst. quoad plant. jap. p.p.

? *Sphagnum rufescens* (N.H.S.) Limpr. quoad plant. jap.

Plant rather robust, normally erect, often submerged and decumbent or procumbent, (3–) 8–15 cm high, often 25 cm long or more in submerged form, normally yellowish brown to reddish brown above, and discolored brown below, more greenish in plants growing in shaded places and dark or purplish brown in water forms, when dried generally with somewhat metalic luster.

Wood-cylinder dark brown in 3–5 layers, with strongly thickened walls and narrow lumens; cortical cells usually in 1–2 layers, accompanying

inner ones with narrower lumen, one layered part increases corresponding to oligocladity, on the surface short or long quadrilateral, commonly with one or two pores or thinnings of wall near the upper end of each cell.

Stem-leaves very variable, normally triangular lingulate to ovate lingulate, strongly concave, (0.90–) 1.00–1.50 (–2.60) mm long and (0.60–) 0.70–0.92 mm wide at the base, the margin involute in the upper part and somewhat cucullate at the apex with hyaline border, the border entire, of 2–5 rows of narrow cells with pitted walls, not or slightly broadened toward the base; auricles rather large. In the isophyllous leaves the apex truncate with 4–5 teeth, but in some peculiar leaves of water form bordered with narrow cells. Hyaline cells normally fibrillose in upper 1/3–2/3 part or down to the base, with few divisions; on the inner surface generally with more numerous pores than on the outer, the pores generally ringed, somewhat larger at the corners of cells and smaller at the upper end and along the commissures, increase in number toward both apical and upper marginal portion and decrease toward the base; on the outer surface normally with a small ringed pore at the upper end and with pseudopores or ringed pores at the corners of cell, often with more numerous ringed pores along the commissures in the apical portion of leaf of isophyllous form.

Branches in fascicles of 3–4 branches, 2–3 of which spreading, thick and smooth, attenuate, falcate, often strongly curved in comal branches, up to 15 mm long, their cortical cells in a layer, retort-cells well differentiated with inconspicuous necks, usually 2 rarely one or 3 in a row.

Branch-leaves broad ovate to ovate lanceolate, often asymmetrical (1.30–) 1.50–2.50 (–4.30) mm long and (0.70–) 0.80–1.00 (–1.80) mm wide, strongly concave, margin involute in the upper part, often somewhat tubular, narrowly or roundly truncate at the apex with (5–) 7–8 teeth, the border narrow, entire, of 1–3 (–4) rows of narrow cells with pitted walls. Hyaline cells fibrillose on both surfaces, vermicular, 10–11 times as long as wide in the central portion, narrower toward the apex to 10–16 times as long, broader toward the base to 6–8 times as long; on the inner surface usually with a few pseudopores or pseudopore-rudiments near corners of cells, rarely with a small ringed pore at the end of cell, occasionally with ringed or bordered pores at both ends and corners of cells in some leaves of plants grown in dried places or in water; on the outer surface commonly with ringed or bordered pores at all angles of cells and with similar pores and pseudopores or pseudopore-rudiments scattered along the commissures, the pores usually increase in number toward the apical and marginal parts and decrease toward the base. Chlorophyll-cells in section rectangular to barrel-shaped with oblong lumens, equally exposed on both surfaces with thick or thin walls.

Dioecious. Antheridia in catkin on spreading branches. Perigonial leaves yellowish brown, shorter than branch-leaves, with obtuse apex, strongly concave, especially near the base, up to 1.50–1.57 mm long and 0.63–0.75 mm wide, slightly differing from the normal branch-leaves by lack-

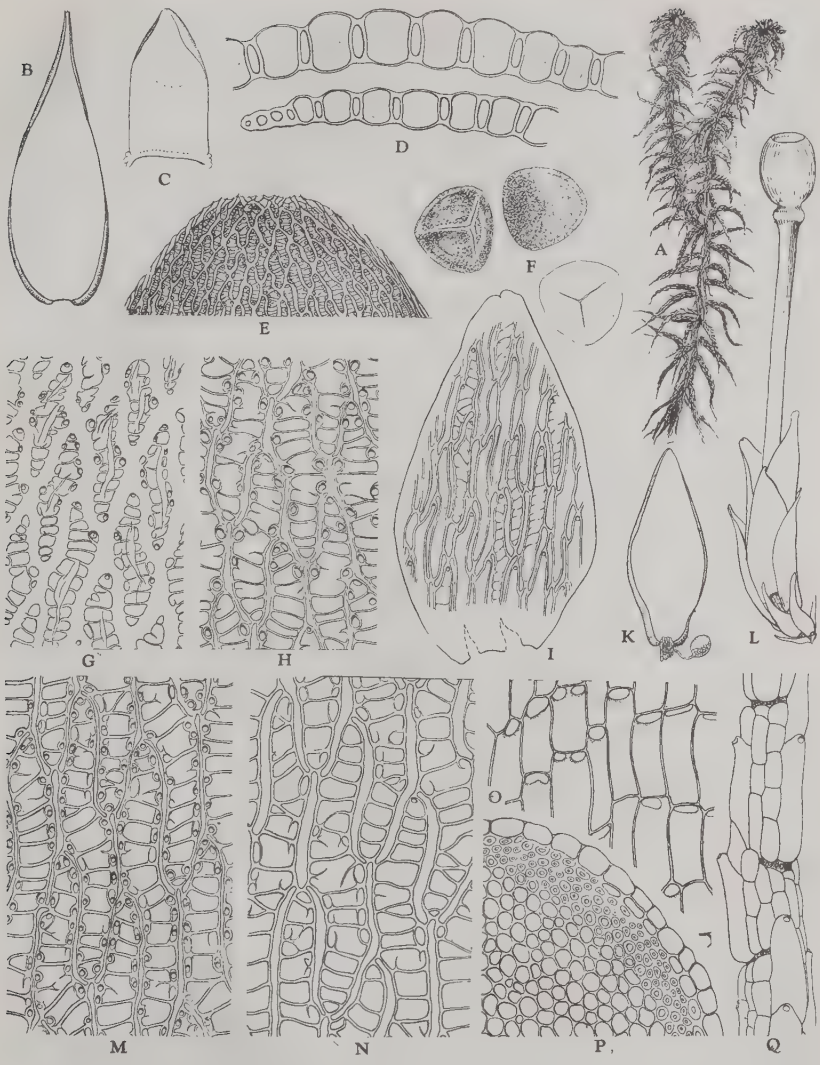


Fig. 10. *Sphagnum subobesum* Warnstorf
A. Sterile plant ×3/4, B. Brach-leaf ×18, C. Stem-leaf ×18, D. Cross-sections of branch-leaf ×335, E. Expanded point of stem-leaf ×167, F. Spores ×335, G. Outer surface of the apical part of stem-leaf ×235, H. Inner surface of ditto ×235, I. Perichaetial leaf ×8 and inner surface of the upper portion of it ×110, K. Perigonial leaf ×18, L. Fruiting branch ×5, M. Outer surface of the central portion of branch-leaf ×335, N. Inner surface of ditto ×335, O. Outer surface of stem ×120, P. Cross-section of ditto ×120, Q. Part of denuded branch ×58. (A, E...H.S. 1278, K...H. S. 10503, the others...H.S. 20300).

ing pores on the outer surface in basal half of the leaf, having usually finer fibrils or often lacking them in a few cells near the base. Fruiting branches usually elongated, erect. Perichaetial leaves up to 13 in number, outer one rather scaly, innermost one largest, 6.0–6.8 mm long and 3.3-3.5 mm wide, ovate, commonly emarginate at the apex, with narrow border of 3-6 narrow cells with indistinctly pitted walls, almost entirely composed of the two kinds of cells. Hyaline cells rhomboidal, 5–7 times as long as wide, almost of similar shape, but slightly narrower toward both apex and base, wholly or scatteredly fibrillose at the upper 1/6 part of leaf, rarely with a few simple divisions; on the outer surface only small end-pores in a few cells in the apical part; on the inner surface end-pores in more numerous cells and in fibrous cells, often some pores occur at the corners besides end-pores.

Capsule elongated sphaerical, 1.5–1.7 mm in diameter and 2.0 mm in height, with small operculum; spores yellow, (36–) 38–39 μ in diameter, often with a large oil body, granular-roughened.

Nom. Jap.: *Shita-mizugoke* (*Uzen-mizugoke*, *Numa-mizugoke* ?, *Kazusa-mizugoke* p. p., *Aka-mizugoke* ?).

Distribution: Endemic to Japan (Hokkaido and Honshu).

Specim. exam.: **Hokkaido**, Prov. Teshio, *Wakkasakinai*, Teshio-gun, Toyotomi-mura, Toyosato～Wakkasakinai, 10 m (H. S. Aug. 18, 1954 H. S. 18802+*S. subsecundum*); *Nayoro*, Kamikawa-gun, Nayoro (Faurie-no. 104, Sept. 1904, as *S. inundatum*, in KYO); Prov. Kushiro, *Oboro*, Kawakami-gun, Shibecha-machi, Tōro, 10 m (H. S. July 22, 1956–H. S. 21211+*S. obtusum*), *Shiranuka*, Shiranuka-gun, Shiranuka-machi, Shoro, (K. Itō, July 25, 1950–H.S. 10390-c), ibid. 5 m (H. S. July 19, 1956–H.S. 21126, 21127 c. fr, 21134); Prov. Tokachi, *Urahoro*, Nakagawa-gun, Toyokoro-mura, Toyokoro, 13 m (H. S. July 23, 1956–H. S. 21225-c, 21227, 21230–21232, 21235, 21236); Prov. Ishikari, *Ebetsu*, Iwamizawa-shi, Horomui plain, 9 m (H.S. Sept. 9, 1952–H.S. 14567); Prov. Iburi, *Oshamanbe*, Yamakoshi-gun, Oshamanbe-machi, Shizukari plain, 7.5 m (H. S. Sept. 11, 1952–H. S. 14593, 14600); Prov. Oshima, *Ōnumakōen*, Kameta-gun, Nanae-mura, Junsai-numa (Faurie-nos. 102, 187, as *S. inundatum*, in KYO).

N. E. Honshu, Prov. Mutsu, Aomori (Faurie-no. 16, Oct. 1902, in KYO & NICH, no. 56, June 18, 1897. isotype of *S. subobesum* in KYO, no. 60 June, 18, 1897, no. 106 May 1, 1897, as *S. inundatum*, in KYO), *Chikagawa*, Shimokita-gun, Higashidōri-mura, Saibana, 15 m (H.S. Sept. 2, 1954–H.S. 19518, 19519), *Ōminato*, Shimokita-gum, Tanabu-machi, Uchita, 5 m (H. S. Sept. 1, 1954–H.S. 19495), *Mutsuyokohama*, Kamikita-gun, Yokohama-mura, Fukkoshi, 10 m (H. S. Sept. 2, 1954–H.S. 19543–19548), *Asamushi*, Higashitsugaru-gun, Uchino-mura, Asamushi, 3 m (H. S. Aug. 31, 1954–H.S. 19441–19445, 19447), *Kanaki*, Kitatsugaru-gun, Kanaki-machi, Ashinokōen, 12 m (H. S. July 28, 1956–H. S. 21302–21304, 21313–21316), *Kawaharadai*, Nakatsugaru-gun, Iwaki-mura, Karekitai, 450 m (H. S. Aug. 12, 1951–H. S. 11902, 11905, 11915, 11916, 11921, 11923, 11925, 11927–11929), *Hirosaki*, ibid. south of Dakeonsen, 430 m (H.S. Aug. 21, 1951–H.S. 11846–11850), ibid. 420 m (H.S. Aug. 12, 1951–H.S. 11860, 11862–11864, 11868, 11869, 11874, 11875, 11878–11880, 11886, 11887, 11895–11897), ibid. Mt. Iwaki, west of Saihōji-mori, 1090 m (H. S. Aug. 11, 1951–H. S. 11818, 11837, 11843); Prov. Rikuchu, *Morioka*, Iwate-gun, Takizawa-mura, Wakare～Yanagizawa, 240 m (H.S. Sept. 14, 1952–H.S. 14916), ibid. Harukoyachi, 460 m (K. Ishizuka & T. Chiba, July 4, 1954–H.S. 19558–19564, 19568, 19569, 19577), Morioka-shi, Iwayama, 300 m (Y. Ikegami-no. 27674, Aug. 7, 1953–H.S. 21729), *Ichinoseki*, Nishiiwai-gun, Itsukushi-mura, Itsukushidaki, (E. Uematsu, June 1, 1907–Okamura no. 28, as *S. okamurae*, in NICH),

Prov. Ugo, *Inaniwa*, Okachi-gun, Minase-mura, Ketakura-numa, 490 m (H. S. Aug. 10, 1953–H.S. 16510, 16511); *Sakata*, Akumi-gun, Minamihirata-mura, Niiyama, Shintame-ike, 50 m (H.S. Aug. 9, 1951–H.S. 11696, 11697 c. anth.); Prov. Uzen, *Uzenkanayama*, Mogami-gun, Mamurogawa-mura, Kamigasawayama, 120 m (H. S. Aug. 2, 1953–H. S. 16183–16185, 16187–16191, 16200–16202), *Naruko*, ibid. Higashioguni-mura, Sakaita, 340 m (H. S. Aug. 11, 1953–H. S. 16532, 16533 c. anth., 16534, 16535, 16539), *Shinjō*, Shinjō-shi, Nashinoki～Yokomai, 150 m (H.S. Aug. 11, 1953–H.S. 16517, 16520–16522), *Gassan*, Higashitagawa-gun, Mt. Gassan, Gōshimizu, 1290 m (H. S. Aug. 23, 1948–H. S. 2801), *Yudonosan*, ibid. Daimanyachi, 380 m (H.S. Aug. 22, 1948–H.S. 3212–3218), *Arato*, Higashimurayawa-gun, Sakuyazawa-mura, Onuma, 600 m (H.S. Aug. 5, 1953–H.S. 16146, 16148, 16149, 16153–16156, 16161–16163, 16170, 16173), *Oguni*, Nishiokitama-gun, Oguni-machi, Hōnoki Pass, 320 m (Y. Ikegami-no. 37326, July 24, 1953–H.S. 20347); *Kaminoyama*, Higashimurayama-gun, Takayuonsen, Dosudaira (Sekine, July 18, 1908 Okamura no. 63, isotype of *S. uzenenze*, in NICH); Prov. Rikuzen, *Sendai*, Sendai-shi (E. Ishiba,-Y. Ikegami-no. 1000, Aug. 22, 1919–H. S. 1612), ibid. Hatadate, 90 m (H. S. Aug. 12, 1953–H. S. 16570–16574, 16576); Prov. Echigo, *Oguni*, Iwafune-gun, Onnagawa-mura, Minaminaka, 80 m (Y. Ikegmai-no. 10784, May 28, 1949–H. S. 7926, 10842), Kitakanbara-gun, Kurokawa-mura, Mochikura, 120–150 m (T. Ozaki-Y. Ikegami-no. 23015, 23019, May 17, 1952–H. S. 20225, 20227), *Nakaj* , Iwafune-gun, Kanaya-mura, Nakano, 9 m (Y. Ikegami-nos. 24777, 24778, Nov. 4, 1952-H.S. 20288, 20286 –no. 36805, May 22, 1955–H.S. 20343), Kitakanbara-gun, Kinoto-mura, Hirakida, 25 m (Y. Ikegami-no. 10234, Sept. 26, 1948–H.S. 4380, –no. 19604, June 3, 1951–H.S. 12561, –nos. 24753, 24754, Nov. 4, 1952–H.S. 20274, 20275, –no. 36786, May 22, 1955–H.S. 20336), ibid. Kurokawa-mura, Kurikinoshinden, 100 m (Y. Ikegami-no. 19596, June 3, 1951–H.S. 12556), Kitakanbara-gun, Tsuiji-mura, Nakamurahama, 5 m (Y. Ikegami-nos. 26511–26514, 26514-b, 26515, June 30, 1953–H. S. 20300 c. fr., 20301–20305), ibid. Sugadani-mura, Mizutani, 160 m (Y. Ikegami-nos. 10925, 10924, June 26, 1949–H. S. 10843, 10844), Kitakanbara-gun, Nakajo-machi, Tossakayama, 60 m (Y. Ikegami-no. 8598, Sept. 22, 1946–H. S. 1644).

M. Honshu, Prov. Echigo, *Shibata*, ibid. Shiunji-mura, Futatsuyama, 3 m (Y. Ikegami-no. 24683, Oct. 4, 1952–H.S. 20243), ibid. Kawahigashi-mura, Kurumanohara, 65 m (Y. Ikegami-nos. 12006–12008, Oct. 15, 1949–H.S. 10762–10764, –nos. 11025, 11026, 11024, 11027, July 9, 1949–H.S. 10845–10848), *Tsugawa*, Higashikanbara-gun, Gejō-mura, Numagoshi Pass, 370 m (Y. Ikegami-no. 8128, Nov. 25, 1945–H.S. 1639), ibid. Tsugawa-machi, Kirinzan, 60 m (Y. Ikegami-no. 24815, Oct. 6, 1952–H.S. 20294), *Niitsu*, Kitakanbara-gun, Sasaoka-mura, Fukui, 20 m (Y. Ikegami-nos. 24698, 24699, 24706, Oct. 11, 1952-H.S. 20251 c. anth., 20252, 20259), ibid. Bunda-mura, Yamamotoshin, 20 m (Y. Ikegami-no. 24691, Oct. 11, 1952–H.S. 20248+*S. microporum*), ibid. Jisha, 20 m (Y. Ikegami-nos. 24687–24689, Oct. 11, 1952-H.S. 20245–20247), *Mikagurayama*, Higashikanbara-gun, Jōjō-mura, Haraikawa, 100 m (Y. Ikegami-no. 9703 May 16, 1948–H.S. 1653, 4371), *Kamo*, Nakakanbara-gun, Nanatani-mura, Kamitakayanagi, 130 m (Y. Ikegami-no. 26352, May 31, 1953–H.S. 20299), ibid. Ko-oto, 200 m (Y. Ikegami-nos. 26331, 26332, May 31, 1953–H.S. 20306, 20307), Minamikanbara-gun, Morimachi-mura, Morimachi Pasture, 80 m (Y. Ikegami-no. 22703, May 10, 1952–H.S. 20223), *Sanjō*. ibid. Shōgawa-mura, Sugizawa, 50 m (Y. Ikegami-no. 30538, June 27, 1954–H. S. 20315), *Nagaoka*, Koshi-gun, Kitadani-mura, Tai, 30 m (Y. Ikegami-nos. 24740, 24741, Oct. 26, 1952–H. S. 20271, 20272), *Okanomachi*, Kariwa-gun, Takeishi, 100 m (Y. Ikegami-no. 9309, Nov. 16, 1947–H. S. 1650), *Ojiya*, Kitauonuma-gun, Koide-machi, Himizo, 200 m (Y. Ikegami-no. 19996, July 25, 1951–H. S. 12571), ibid. Imegasaki-mura, Haramushino-shinden, 100 m (Y. Ikegami-no. 19956, July 24, 1951–H. S. 12568), ibid. 150 m (S. Ōdaira-Y. Ikegami-no. 25128, Sept. 20, 1952–H. S. 20298), ibid. Hirokami-mura, Takinomata, 480 m (Y. Ikegami-no. 42159, June 6, 1956–H. S. 21820); Prov. Sado, *Aikawa*, Sado Isl., Sado-gun, Kinpokusan, 700 m (Y. Ikegami-no. 376, June 9, 1935–H. S. 1609), ibid. on Ryōtsu

course, 1000 m (K. Homma-Y. Ikegami-no. 20113, June 24, 1951–H. S. 21724, K. Suda-Y. Ikegami-no. 22817, July 25, 1952–H. S. 20224); Prov. Iwashiro, *Fukushima*, Shinobu-gun, Ōzaso-mura, Jūroku-numa, 120 m (R. Watanabe, Dec. 13, 1953–H. S. 17640), *Ba daisa* ?, Yama-gun, Hibara-mura, Onezawa~Oguni-numa, 800–1000 m (H. S. Aug. 24, 1950–H. S. 10473), ibid. Oguni-numa, 1000 m (H. S. Aug. 24, 1950–H. S. 10474, 10488, 10490, 10500, 10503, 10508, 10513, 10514, 10516, 10517, R. Watanabe, Aug. 4, 1954–H. S. 19645), Kitaaizu-gun, Minato-mura, Akaiyachi, 530 m (S. Miki-R. Toyama-no. 1798, Oct. 27, 1927–H. S. 17487, Y. Ikegami-no. 1474, July 25, 1940–H. S. 1617, Y. Ikegami-no. 28989, Aug. 26, 1953–H. S. 21763 c. fr. & anth.); *Nozawa*, Kawanuma-gun, Nozawa-machi, Nishidaira, 220 m (Y. Ikegami-no. 44333, Oct. 8, 1956–H. S. 21852 c. fr.); *Sukagawa*, Iwase-gun, Kagamiishi-mura, Kasaishi, 260 m (H. S. July 29, 1956–H. S. 21336, 21337); Prov. Iwaki, *Sukagawa*, Nishishirakawa-gun, Yabuki-machi, Yabuki~Izumizaki, 290 m (Y. Ikegami-nos. 34639–34643, Aug. 14, 1954–H. S. 21788–21792), ibid. Ōike, 280 m (H. S. July 29, 1956 H. S. 21318–21320, 21322–21325), ibid. Mikami-mura, Kanta, 280 m (H. S. Aug. 22, 1951–H. S. 12335), *Tanagura*, Nishishirakawa-gun, Kawasaki-mura, Jukkenmai, 280 m (H. S. Dec. 8, 1947–H. S. 1281, 1283, 1284–1298, 1300), ibid. 290 m (H. S. Dec. 8, 1947–H. S. 1249, 1251–1280), ibid. Muguro-uchi~Jukkenmai, 300 m (H. S. Dec. 8, 1947–H. S. 1315, 1316), ibid. Mugurouchi, 300 m (H. S. Dec. 8, 1947–H. S. 1241 c. anth., 1242–1244), ibid. north of Yorii, 300 m (H. S. Aug. 24, 1951–H. S. 12360, 12361), ibid. Sekihira-mura, Kagenohara, 290 m (H. S. Dec. 8, 1947–H. S. 1233, 1234, 1235 c. anth.), ibid. Karasugawa, 290 m (R. Watanabe, Sept. 10, 1951–H. S. 12905); *Shirakawa*, Nishishirakawas-gun, Shirasaka-mura, near Station, 400 m (Y. Ikegami-no. 34555, Aug. 12, 1954–H. S. 21785), Shirakawa-shi, Nanko Park, 350 m (H. S. Dec. 4, 1947–H. S. 1182, 1183); Prov. Noto, *Ōchigata*, Hakui-gun, Ōchi-machi, Nakagawa (J. Satomi, Oct. 20, 1950–H. S. 10755); Prov. Shinano, *Suwa*, Suwa-shi, Mt. Kirigamine, Yashimagahara moor, (Y. Kubota-no. 317, July 1, 1950–H. S. 20390), ibid. southern part of the moor, 1640 m (H. S. July 26, 1954–H. S. 18160-a, 18161–18163, 18170, 18171, 18174–18176), ibid. Yashimaga-ike, 1650 m (H. S. July 13, 1949–H. S. 5801–5803), ibid. Ko-ike, 1650 m (H. S. July 13, 1949–H. S. 5828), ibid. Kamaga-ike, 1650 m (H. S. July 13, 1949–H. S. 5863–5866, 5868), ibid. Odoriba moor, 1560 m (H. S. July 14, 1949–H. S. 5900, 5902), ibid. Kurumayama moor, 1760–1780 m (T. Seki-nos. 6669, 6679, 6670, Aug. 15, 1956–H. S. 21610, 21611+*S. microporum*, 21671+*S. microporum*), ibid. 1850 m (H. S. July 14, 1949–H. S. 5933-b, Y. Kubota-no. 256, June 30, 1950–H. S. 20377).

There are approximately 84 known localities of this species in Japan; viz. 8 in Hokkaido, 34 in N.E. Honshu and 42 in M. Honshu. The majority of the localities in M. Honshu are situated in its northern parts and the majority in Hokkaido are located in its southern half. It is limited to lowlands of less than 20 m in elevation in Hokkaido, ascending to over 1,000 m in elevation in northern Honshu. According to the writer's present knowledge, the northernmost limit is between Toyosato and Wakkasakinai (45° 07′ N.L.) located in the northern part of Hokkaido, and the southernmost limit is Mt. Kirigamine (36° 07′ N.L.) located in M. Honshu. The highest elevation of these localities is Kurumayama moor on Mt. Kirigamine where it ascends to 1,850 m and the lowest is Asamushi located near the northern end of Honshu and Shiunji-mura situated in the coastal plain of M. Honshu on the Japan Sea, where it descends to about 3 m above sea-level. From the range of its vertical and horizontal distributions, it may be said that this species has its center of distribution in northern Honshu.

8. Sphagnum platyphyllum (Sull. ex Lindb.) Warnstorf, Flora **67** : 481. (1884) ; H. Suzuki, Journ. Sci. Hiroshima Univ. Ser. B. Div. 2. **7** : 84. *f.* 5. (1955).

Nom. Jap.: *Hiroha-mizugoke.*

Distribution: Scandinavian Peninsula, Central Europe (in the Alps up to 2,000 m alt.), Atlantic Europe (Portugal), Balkhan Peninsula, Russia (Arctic, European regions, Caucasia, Western & Eastern Siberia and Far East), Japan (Hokkaido) and Atlantic North America.

Specim. exam.: **Hokkaido,** Prov. Ishikari, *Iwamizawa,* Bibai-shi, Bibai plain, north of Nimaibashi, 13–14 m.

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